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SOIL SCIENCE

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THE NON-BIOLOGICAL OXIDATION OF ELEMENTAL SULFUR IN QUARTZ MEDIA

A Preliminary Report

W. H. MacINTIRE, F. J. GRAY AND W. M. SHAW

University of Tennessee Agricultural Experiment Station

Received for publication December 24, 1920

The conversion of native organic sulfur into sulfates in soils is generally considered to be almost exclusively a biological process. The oxidation of added elemental sulfur is likewise usually attributed to the action of bacteria. The native organic sulfur phase of sulfate generation, as influenced by calcic and magnesic materials in varying amounts, has been under investigation at the University of Tennessee Agricultural Experiment Station since July, 1914. At that time, 46 lysimeters were installed and filled with Cumberland loam, 23 tanks having soil alone, and 23 having surface soil above a 1-foot layer of clay subsoil. Each annual aggregate of sulfate leachings has been determined quantitatively. Divergent effects of calcic and magnesic compounds upon the sulfate outgo, during the first two years, were noted and reported upon in a preliminary paper by the writer and associates (2) in 1917. The supplemental problem of sulfur additions to a Cherokee sandy loam was begun in August, 1917. Fifteen tanks received sulfur additions. Each five-tank group received one of the three forms of sulfur, namely; iron sulfate, iron pyrite, and flowers of sulfur, each in an amount equivalent to a rate of 1000 pounds of sulfur per 2,000,000 pounds of soil. The question of the influence of lime and magnesia upon added sulfur was also included in the supplementary study. In this second installation, comprising a total of 22 lysimeters, the loss of sulfur, as leached sulfates, was determined for each tank periodically, as necessitated by the unsupplemented rainfall. The data secured from the leachings demonstrated that the flowers of sulfur and iron pyrite were both converted into sulfates, with distinct rapidity.

It was at first assumed that the oxidation of both the elemental sulfur and that of pyrite was induced in the main, if not solely, by organisms. However, some doubt was introduced concerning this assumption about two years after the inauguration of the experiment. At that time it was observed that a strong odor of SO_2 was given off from the reserve sample of iron pyrite. This reserve sample had been kept in the dark in an 8-ounce glass bottle, tightly stoppered with an ordinary no. 6 cork stopper. Examination of the pyrite showed that it contained a large amount of SO_4. A 10-gm. charge was found to yield soluble sulfate of iron, equivalent to a determined weight of 0.4172 gm.

of $BaSO_4$, as an average of seven determinations. A search of the literature revealed that the same observation had been made by Allen and Johnston (1) reported in 1910. These workers further reported that an increase of 100 per cent of sulfate of iron was caused by dry grinding for a period of 1 hour. They accounted for the reaction through the following equation:

$$FeS_2 + 3\ O_2 \rightleftarrows FeSO_4 + SO_2$$

Contact of moist pulverized metallic iron and flowers of sulfur was found to produce iron sulfide, which reaction was also found to be of record (3). These observations suggested the possibility that the applied elemental sulfur might combine to an extent, with the iron of the soil, forming iron-sulfur compounds which, in turn, would undergo oxidation to sulfates. It even seemed plausible to assume that the presence of iron in some form might be essential to the extensive conversion of elemental sulfur into sulfates.

These findings and observations led to a laboratory study of the two major queries:

1. What function, if any, does metallic iron, and what function does iron oxide, or oxides, have upon the conversion of elemental sulfur to sulfates in soils?

2. Will the effects possibly induced by iron, or its oxides, be independent of biological activation?

It was, therefore, planned to study the oxidation of elemental sulfur in the absence of appreciable quantities of iron, under aerobic and anaerobic conditions, with the unaltered medium, the sterilized medium, and the medium plus inoculation. It was decided to use the purest obtainable quartz as the medium for sulfur additions. An unsuccessful attempt was made to secure an iron-free quartz. The finely ground New England quartzite utilized ran 99.28 per cent SiO_2, 0.34 per cent Fe_2O_3 and 0.0096 per cent S. The purest hydrogen-precipitated Fe obtainable was used as one source of iron. This material carried 0.0475 per cent of sulfur. The other iron compound used was limonite, analyzing 39.50 per cent iron and 0.013 per cent soluble sulfate sulfur. A futile attempt was made to obtain a sufficient quantity of siderite as another source of iron.

Five-hundred cubic centimeter Pyrex flasks were used as containers for the treated media. The unleached quartz was used in the constant amount of 250 gm., with 14 per cent distilled water additions for moisture. Each treated medium was kept in the dark for a period of 60 days after treatment. The quartz was very finely ground. In addition to the constant amount of 250 gm. of quartz, the following respective single or combined constants were used: 0.1251 gm. of sulfur; 10.0806 gm. of metallic iron; 25.3164 gm. of limonite; 0.5076 gm. of "c.p." precipitated calcium carbonate; 0.5000 gm. of "c.p." precipitated magnesium carbonate; 0.5181 gm. of 100-mesh limestone; and 0.5449 gm. of 100-mesh dolomite. The calcic and magnesic materials were chemically equivalent and were introduced in order to prevent the accumu-

lation of any generated acids, rather than upon the assumption of any direct influences.

The biological conditions maintained in the original quartz-medium experiment were: (a) unaltered quartz, (b) quartz sterilized by heat, (c) inoculation by soil infusion "A," (d) inoculation by soil infusion "B." These four conditions were maintained under both aerobic and anaerobic conditions. The aerobic flasks, both sterile and non-sterile, were stoppered with cotton plugs. The anaerobic atmosphere was produced by a 6-hour passage of purified CO_2. An additional series containing purified hydrogen also was subjected to experimental treatment, but this series is not yet ready for detailed report. However, it may be stated that the formation of sulfates, in considerable amounts, has occurred in the hydrogen atmosphere.

The sterilization was effected by three successive daily heatings in the autoclave, without contact of quartz and the separately sterilized materials used in the several treatments. The added materials were mixed throughout the dry quartz immediately before the addition of the constant moisture content. All of the flasks were put away in the dark, in a room relatively free of fumes, for a period of 60 days. At the end of the 60-day period the contents of the flasks were extracted by addition of cold distilled water to near-complete flask volume. After 4 hours' shaking and over-night standing, the extracts were filtered through Büchner funnels with double filters. Each residue was then thoroughly mixed and returned to its original flask for an additional period of contact of 40 days, after which the filtration was repeated. The filtrates were analyzed for sulfides, and if deemed necessary, NaOH was introduced. They were then acidified and evaporated to dryness, in order to remove SiO_2. The engendered sulfates, as well as the sulfates of all blanks, were determined gravimetrically. Tests were made to insure the fact that the precipitations were not BaF_2. In addition to the eight series of 15 flasks each, one additional set was run simultaneously. This supplemental set contained 12 flasks. Three flasks contained inoculated quartz and nitrate nitrogen to the extent of 10 mgm. of nitrogen, one flask containing sodium nitrate, one calcium nitrate, and one magnesium nitrate. These three nitrate treatments were duplicated with an increase of nitrate nitrogen to a basis of 50 mgm. The six flasks, above described, were then duplicated as to nitrogen treatment, but with the addition of 10.0806 gm. of metallic iron to each flask.

The different treatments given in each of the 15-unit sets, with both aerobic and CO_2 atmospheres and under the four conditions of moist contact, original quartz, sterilized quartz, quartz with inoculation "A" and quartz with inoculation "B," are listed below.

Sulfur alone

Sulfur plus $CaCO_3$
Sulfur plus limestone
Sulfur plus $MgCO_3$
Sulfur plus dolomite

Sulfur plus metallic iron
Sulfur plus iron plus $CaCO_3$
Sulfur plus iron plus limestone
Sulfur plus iron plus $MgCO_3$
Sulfur plus iron plus dolomite

Sulfur plus limonite
Sulfur plus limonite plus $CaCO_3$
Sulfur plus limonite plus limestone
Sulfur plus limonite plus $MgCO_3$
Sulfur plus limonite plus dolomite

It is not intended, at this time, to enter into a detailed discussion of the results. However, a few of the salient findings will be mentioned. In each one of the sixty aerobic treatments there was evidenced a substantial increase in leachable sulfates. The four quartz-sulfur-only checks gave an average increase of 222.5 pounds of sulfate sulfur per 2,000,000 pounds of solid medium, for the four conditions with access to the atmosphere. The four calcic and magnesic materials supplementing sulfur additions in the first group, gave an increase of 396.3 pounds of leached sulfur, as an average of sixteen determinations for the four conditions of the medium. The depressive tendency of metallic iron was evidenced in all of the twenty flasks of the second aerobic group. The average increase in leached sulfate sulfur from these five treatments, under the four experimental conditions, which involved twenty flasks, amounted to 109.5 pounds per 2,000,000 pounds of medium. In the third aerobic group, wherein all twenty flasks contained limonite, an average increase of 475.2 pounds of leached sulfate sulfur was obtained, as calculated to a basis of 2,000,000 pounds of medium. When averaged, the fifteen flasks, to all of which sulfur was added either alone or supplemented by the specified supplementary materials, gave increases of sulfate sulfur amounting to 418.9 pounds, 184.5 pounds, 331.1 pounds, and 329.2 pounds, respectively, for the unaltered quartz, the heat-sterilized quartz and the quartz which received two separate inoculations, "A" and "B."

In the CO_2-atmosphere series, the four flasks containing only sulfur and quartz gave an average increase of 147.3 pounds of sulfate sulfur from the four experimental conditions of contact. The sixteen quartz-sulfur-carbonate flasks yielded an average increase of soluble sulfate sulfur amounting to 208.9 pounds per 2,000,000 pounds of quartz. A very striking set of results was obtained from the metallic iron group under the anaerobic conditions. In each one of the twenty flasks, a minus quantity of sulfates was recovered. That is, the amount recovered in each instance was actually less than in the corresponding checks of quartz alone and quartz plus the single or combination treatments, including sulfur. When averaged, the twenty consistent minus quantities, ranging between -8.2 pounds and -50.8 pounds, gave -30.3 pounds as representing the depressive action of the metallic iron. The third, or limonite, group in the sealed CO_2 atmosphere gave, as an average of

five flasks for each of the experimental conditions, 305.4 pounds, 319.8 pounds, 126.0 pounds and 139.0 pounds for the unaltered quartz, sterilized quartz and quartz with inoculations "A" and "B," respectively. As an average of the entire fifteen flasks receiving the different additions, under each of the four experimental conditions, the increased outgo of sulfate sulfur amounted to 184.5 pounds, 134.5 pounds, 83.4 pounds, and 95.3 pounds, respectively, for the unaltered quartz, the sterilized quartz and the quartz with inoculations "A" and "B." Corresponding increases of 295.6 pounds, 241.7 pounds, 139.4 pounds and 157.7 pounds were obtained by the omission of the several constant minus results obtained from the metallic iron group; that is, averaging only the increases, which were obtained in every case in the absence of metallic iron.

In the case of the supplementary inoculated CO_2-atmosphere nitrate series, the smaller nitrate additions failed to show any extensive increase in leached sulfates, above that given by the quartz-sulfur checks, after a 60-day period of contact. On the other hand, the larger nitrate additions exhibited a distinct and consistent depressive influence for each of the three added nitrates. Again in the six flasks containing nitrates and metallic iron, the same characteristic depressive tendency of iron was demonstrated. After the leaching of the nitrates, along with the soluble engendered sulfates, a further contact period of 40 days gave a decided gain in sulfates where both quantities of nitrate nitrogen were added alone; but the minus-quantity characteristic of the metallic iron treatment still obtained in those flasks where both nitrates and iron were introduced.

These results appear positively to establish certain facts. Elemental sulfur will oxidize upon moist contact with relatively pure quartz under both aerobic and anaerobic conditions, as represented, respectively, by access to air through cotton plugs and contact with a sealed atmosphere of purified CO_2. A distinct reducing and inhibitive effect is manifested by metallic iron under aerobic conditions, with a still greater similar tendency in the atmosphere of CO_2. Limonite, on the contrary, exhibits a strong tendency to accelerate oxidation when used alone and when supplemented by the several carbonates. Furthermore, the single additions of the several carbonates to the quartz-sulfur mixture resulted in a marked increase in the amounts of sulfates recovered under the aerobic conditions.

In the case of the supplementary nitrate series, the presence of the several nitrates failed to alter the depressive tendencies of metallic iron. In fact, the increase in salt-concentration exhibited an independent depressive action in the absence of the metallic iron. This observation relative to the influence of the concentration of salts in the moisture of the several media, parallels that of the depressive tendency of precipitated $MgCO_3$, when its solubility in water was materially increased through contact with an excess of CO_2.

The chemical explanations of the several observed phenomena will be considered in the more detailed papers to be offered shortly. However, it might

be pointed out at this time that oxygen is readily furnished to the added elemental sulfur through the agency of limonite and through contact with each of the four separate carbonate treatments, where access to air is permitted through cotton plugs. Were the oxidation assumed to be caused as a result of aeration, the added treatments would still necessarily be considered as accelerators. However, the same tendencies have been found to manifest themselves in atmospheres of purified CO_2 and purified hydrogen, which would militate against the aerobic assumption. Again, in the anaerobic flasks, oxygen is available only from water, SiO_2, limonite, carbonates and the CO_2 gas; unless it be assumed that there occurs upon the surface of the quartz a condensed film of oxygen, or air, not replaced by CO_2 gas, nor by hydrogen; but, nevertheless, available for oxidation of the elemental sulfur under conditions of intimate moist contact. An effort is now being made to obtain some light upon this point. Studies are being made also upon hypothetical by-products such as carbon monoxide, hydrogen, ferrous oxide and others.

It should be stressed that our experiments were not designed to demonstrate that native organic soil sulfur, or added elemental sulfur, is not converted into sulfates through biological activities. Rather, an attempt was made, to determine, as it appears to be definitely established, whether the oxidation of elemental sulfur into sulfates may be induced solely by chemical reactions within a siliceous medium, or media; and, furthermore, what part iron and iron oxide may have in inducing the reactions both alone and with supplementary alkali-earth carbonates.

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THE AMOUNT OF UNFREE WATER IN SOILS AT DIFFERENT MOISTURE CONTENTS

GEORGE BOUYOUCOS

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INTRODUCTION

It has been shown that the results yielded by the freezing-point (2) and dilatometer (1) methods reveal a most intimate and complex relationship between soil and water and necessitate a new classification of the soil moisture on a more logical, real and scientific basis. The new classification that has been revealed by the results of the above methods is as follows:

Gravitational water
Free water
Unfree water { capillary-adsorbed
combined { water of solid solution or
water of hydration

The free water is that which freezes readily at slightly below zero; while the unfree water is that which freezes finally at the supercooling of −4°C. and also in the temperature of −78° minus the free water. The capillary-adsorbed water, which is part of the initial total unfree water, may become free by certain treatments of the soil, notably repeated freezing and thawing, while the combined water, which is also part of the total unfree water, seems not to be affected by such treatments.

When the freezing-point lowering of soils is determined at different moisture contents it is found that the freezing-point depression increases at an abnormally greater rate than the moisture content decreases. Indeed, the results show that the depression tends to increase in a geometric progression as the moisture content decreases in an arithmetic progression. These facts would seem to indicate that the amount of unfree water increases as the total moisture content decreases. In offering originally an hypothesis to explain the results, it was postulated, however, that the amount of water which the soils cause to become unfree is rather definite at any one moisture content, although part of this unfree water can be rendered free by successive freezing and thawing. This postulate seemed later to be verified by the dilatometer method results which showed that the amount of water which failed to freeze in any one soil was practically the same irrespective of the moisture content.

Keen (3) of the Rothamsted Experiment Station, in a very thorough and intelligent critique of the results yielded by the freezing-point and dilatometer methods together with those that he obtained himself on the evaporation of water in soils, has pointed out that according to mathematical treatment of the experimental data the amount of water rendered unfree by the soil appears not to be constant but varies with the total moisture content. He has applied mathematics to the experimental data and has arrived at the conclusion that the proportion of free to total water continually decreases and that of unfree to total continually increases as the total moisture diminishes in amount. This conclusion is contrary to the original postulate proposed, which appeared to be confirmed by experimental data.

Keen is to be highly commended in his efforts to treat experimental results on a quantitative basis, for the more we can express experimental data in soils mathematically the more we may be able to reduce the subject of soils to an absolute science. Unfortunately, however, soil is such a heterogeneous and complex mass with so many unknown and unforeseen factors that it is not always safe to arrive at or predict conclusions mathematically.

Since a correct knowledge on the subject of the quantity of unfree water in soils at different moisture contents is of considerable theoretical interest as well as of practical importance, and in view of the fact that the mathematical consideration of the experimental data by Keen indicate conclusions the opposite from those published by the writer, it was decided to reinvestigate experimentally the whole problem more extensively and thoroughly than it had been done before. In the following pages, therefore, are presented the experimental results obtained and the conclusions to which they point.

AMOUNT OF UNFREE WATER IN SOILS AT DIFFERENT MOISTURE CONTENTS

The method employed for ascertaining whether the amount of water rendered unfree by the soil varies with the different moisture contents or remains constant, was the dilatometer method.[1] The procedure consisted of placing soil of different moisture contents in the dilatometer and determining the amount of water that refused to freeze at different temperatures. Two different temperatures or supercoolings were employed: $-1.5°$ and $-4°$C. The object of employing these two temperatures was to obtain data both for the total unfree water and for the combined water. As previously stated all the water that refuses to freeze for the first time at the supercooling of $-1.5°$C. is designated as unfree water and is composed of capillary-adsorbed and of combined water. The capillary-adsorbed water will finally freeze at the temperature of $-4°$C., but the combined will not even at the extreme low temperature of $-78°$.

By determining, therefore, both the total unfree and combined water the question as to whether the amount of water rendered unfree by the soil varies

[1] For detailed description of procedure and method see Bouyoucos (1).

with different moisture contents or remains constant, can be more conclusively answered.

In table 1 are presented the experimental results obtained. They show both the total unfree and combined water for a large number of soils at different moisture contents. Both the total unfree and combined water are expressed in percentages based only on the water added and on the air-dry soil. The water content of the soil represents only the water added and does not include the hygroscopic moisture. The hygroscopic moisture was not taken into consideration in the calculation of these results, first because it is desired to emphasize the fact that soils are able to cause water to become unfree even above the hygroscopic moisture, and second because from the practical standpoint it is the moisture above the hygroscopic point that is of importance.

From these results it becomes conclusively evident, therefore, that the amount of water which the soils are able to render unfree does not vary with different moisture contents but appears to remain constant. It will be readily seen that both the total unfree and combined water are practically the same at all the different moisture contents and in all the various classes of soil. There is a very slight tendency for both of these forms of water to be greater at the higher moisture contents, but this is largely due to the fact that at the higher moisture contents all the soil particles are more thoroughly and completely wetted and consequently the full amount of water is rendered unfree, and partly also to the fact that it is more difficult to expel all the air from the very wetted soils and this causes a slight error.

These experimental results, therefore, fully confirm the original suggestion made, namely, that the amount of water the soils cause to become unfree appears to be definite, and consequently they disagree with the conclusion derived mathematically by Keen. Evidently there are some unknown factors coming into play in the actual experimental results which are not taken into consideration in their mathematical treatment.

Although the total amount of unfree water seems not to vary at the different moisture contents it does appear, however, to vary with changes in the physical condition of the soil. As has already been stated the process of repeated freezing and thawing tends to liberate some of the unfree water and thereby diminishes considerably its original amount. Other treatments, such as coagulating or deflocculating the colloidal material in the soil by chemical agents, also may cause a variation in the total unfree water. The form of water which is susceptible to this change is the capillary-adsorbed. The combined does not appear to vary with physical changes in the soil, but seems to remain constant.

Although the free, capillary-adsorbed and combined water in the soil, as classified by the dilatometer method, exists in distinct forms, the transition from one into the other is not absolutely sharp. Some of the capillary-adsorbed water which is only weakly held by the forces of the soil is com-

TABLE 1

Amount of water rendered unfree by soils at different moisture contents, hygroscopic moisture not included

Coarse sand						
Moisture content, per cent	5.00	7.50	10.00	12.50	15.00	75.00
Total unfree water, per cent	1.21	1.52	1.60	1.42	1.51	1.62
Combined water, per cent	0.82	0.68	0.92	0.63	0.84	1.03
Medium fine sand						
Moisture content, per cent	5.00	7.50	10.00	12.50	15.00	75.00
Total unfree water, per cent	1.84	1.98	1.57	1.83	1.93	2.16
Combined water, per cent	1.25	1.37	1.18	1.45	1.43	1.51
Fine sandy loam						
Moisture content, per cent	7.50	10.00	12.50	15.00	17.50	75.00
Total unfree water, per cent	4.24	4.18	4.36	4.52	4.45	4.56
Combined water, per cent	2.43	2.18	2.63	2.34	2.28	2.78
Loam						
Moisture content, per cent	12.50	15.00	17.50	20.00	75.00	
Total unfree water, per cent	8.56	8.17	8.73	8.38	8.82	
Combined water, per cent	4.51	4.35	4.16	4.72	4.94	
Silt loam						
Moisture content, per cent	12.50	15.00	17.50	20.00	75.00	
Total unfree water, per cent	9.75	9.35	9.52	9.81	9.84	
Combined water, per cent	5.28	5.42	5.37	5.61	5.45	
Silty clay loam						
Moisture content, per cent	15.00	17.50	20.00	22.50	75.00	
Total unfree water, per cent	13.62	14.03	13.85	14.04	14.25	
Combined water, per cent	7.54	7.61	7.28	7.73	7.92	
Clay loam						
Moisture content, per cent	15.00	17.50	20.00	22.50	75.00	
Total unfree water, per cent	13.13	12.85	12.87	13.24	13.61	
Combined water, per cent	7.28	7.42	7.17	7.04	7.58	
Humus clay loam						
Moisture content, per cent	17.50	20.00	22.50	25.00	75.00	
Total unfree water, per cent	15.35	15.82	15.18	15.61	16.34	
Combined water, per cent	8.73	8.22	9.05	8.82	9.18	
Clay						
Moisture content, per cent	20.00	22.50	25.00	27.50	75.00	
Total unfree water, per cent	14.65	15.18	14.91	15.61	15.45	
Combined water, per cent	9.08	9.23	9.35	9.45	9.68	
Clay						
Moisture content, per cent	20.00	22.50	25.00	27.50	75.00	
Total unfree water, per cent	15.24	15.46	15.18	15.62	15.85	
Combined water, per cent	10.54	10.65	10.56	10.81	11.18	
Muck						
Moisture content, per cent	80.00	100.00	120.00	140.00	200.00	
Total unfree water, per cent	63.82	64.15	65.16	64.23	65.28	
Combined water, per cent	42.53	42.35	42.91	43.28	44.19	
Peat						
Moisture content, per cent	80.00	100.00	120.00	140.00	200.00	
Total unfree water, per cent	69.23	70.15	69.18	70.35	71.13	
Combined water, per cent	51.28	51.93	52.18	52.62	53.95	

pelled to freeze as free water by the force of freezing or crystallizing. A small part of the capillary-adsorbed water which exists in the most intimate contact with the soil and is held with the greatest force, probably does not freeze. Hence, the curves showing the rate of evaporation of water, and the freezing-point depression at different moisture contents, would not reveal any sudden breaks.

SUMMARY

In this paper are presented experimental results showing that the amount of water which the soils are able to render unfree does not vary with the different moisture contents but that it appears to remain constant.

This knowledge is of much importance, especially in relation to the availability of moisture in soils at different moisture contents, the possible behavior of soils toward fertilizers, the physical condition of the soil, etc.

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LIME REQUIREMENT AND REACTION OF LIME MATERIALS WITH SOIL

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The relative value of different lime materials has been determined in various ways. Large-scale field experiments conducted by a number of stations have afforded reliable information as to the effects upon crop yields to be expected after an application of the materials commonly used for liming. Numerous pot experiments have furnished indications of the effect of soil amendments never used in practice, but which are considered to be of interest from a theoretical point of view. The action of these materials has been explained from the effect upon the growth of particular crops, upon the formation of nitrates, upon the soil reaction as shown by various tests, and from the amounts of carbonate and total calcium and magnesium residual or found after intervals. In very few instances have the various proposed lime-requirement methods been considered sufficiently reliable to be of service as indicators of comparatively small changes brought about by the addition of basic materials to the soil.

The experimental work discussed in this paper was undertaken primarily to determine the adaptability of the vacuum lime-requirement method (2) to the measurement of differences in amounts of bases absorbed by soil. A secondary consideration was the comparison of the rates of reaction with soil, or relative "availability" of limestone and other basic materials. In a previous report (2) from this laboratory, it was stated that of the several methods tested, the vacuum method alone gave much promise, because it was satisfactorily quantitative in its indications and seemed to be universally applicable. Knight (7) made a comparison of results obtained by a lime-requirement method in which the hydrogen electrode was used, with those obtained in this laboratory by several methods, with the same soils. He found that the indications of his electrometric method and the vacuum method were substantially in agreement. Stephenson (12) has endorsed the principle of the method, i.e., the use of pure calcium carbonate and water only for measuring the soil's reactivity, but criticises the use of any heat whatsoever. On this phase of the subject, the present work has some bearing.

The comparative rates of reaction or absorption of bases by soil constituents from a high-calcium, nearly pure limestone in five different degrees of fineness have been determined. Calcite (Iceland spar), magnesite and dolomite ground to pass a 100-mesh sieve, and the chemically prepared oxides and

carbonates of calcium and magnesium,[1] and two industrial slags,[2] in a comparable state of comminution, have been compared with the limestone. The rates of decomposition of finely divided limestone in one alkaline and two acid soils are compared. The conclusions reached have been based upon data obtained from carbonate determinations at stated intervals, and from lime-requirement determinations. In some instances, these data have been supplemented by determinations of accumulated nitrates.

The experiment here described was of a preliminary nature, and some of its features, in the light of later knowledge, were not perfect. Certain precautions in the matter of preparation of samples were shown by experience to be essential to the best results. The disturbing influence of nitrification was not sufficiently appreciated. At the same time, an account of the work may be of help to others, while the general results seem to be of sufficient interest and importance to warrant publication.

PLAN OF EXPERIMENT

A large amount of soil was obtained from the edge of one of the fertility experiment fields at the Ohio Agricultural Experiment Station. Although the soil was supposed never to have been limed, it is possible that it had at some time received a very light application when limestone intended for another part of the field was hauled over this part. The carbonate content of the soil is very small, but still distinctly larger than is found in the soil of the unlimed areas of the station farm. Otherwise, it was typical Wooster silt loam, acid to litmus paper and the Truog test (14). The surface soil only, to the depth of 7 inches, was taken. After partial drying, it was sifted through a screen of 2-mm. mesh, all the coarser material being rejected. Single pots also were prepared, one with an acid clay loam soil of the Trumbull series from one of the station's experimental fields at Strongsville, the other with an alkaline silty clay loam of the Clyde series, from the Paulding County experimental farm. These latter soils had been in storage for a year or more and were air-dry. Both were sifted in the same manner as the Wooster soil.

[1] The magnesium compounds were the U. S. P. "light" variety.

[2] The material designated "Slag (Al)" was supplied by the Electric Smelting and Aluminum Co., Sewaren, N. J. It is the *dicalcium silicate* discussed by Hartwell and Pember (6), in relation to its influence upon plant growth. "Slag (B.F.)" is blast furnace slag. The analyses of these slags were stated by the manufacturers to be as follows:

	Slag (Al)	*Slag (B.F.)*
SiO_2	28.7	31.6
Al_2O_3	6.3	14.9
Fe_2O_3	1.9	—
CaO	46.8	36.9
MgO	2.9	15.2
Na_2O	6.5	—
CO_2 and H_2O	6.9	—
S	—	1.8

Unless otherwise stated, the materials to be mixed with the soil were ground to pass through a sieve having 100 meshes to the linear inch. They were added at the rate of 0.310 per cent pure calcium carbonate in the air-dry mixture in the case of the pots with Wooster soil and 0.304 per cent pure calcium carbonate in the air-dry mixtures of Strongsville and Paulding soils. The actual amounts of the several amendments to furnish bases equivalent to the specified percentages of calcium carbonate were determined by titration in a manner similar to that described by Conner (4), except in case of the slags. These were applied at such a rate as to carry calcium and magnesium equivalent to the other applications. It should be noted, however, that both slags were almost entirely soluble in normal hydrochloric acid.

Portions of soil of 11.34 kgm. each were weighed into a large container, then transferred to another, a little at a time, the lime material being gradually added and thoroughly worked in with the hands to avoid lumps. The mixture was then poured back and forth ten times, passing through a screen of 1-cm. mesh each time, and finally placed in a stoneware crock 20 cm. in height by 25 cm. in diameter. Here it was thoroughly compacted and 2 liters of distilled water added. An attempt to simulate natural conditions was made, although there was no crop on the pots. To this end, the pots were neither excessively watered nor permitted to dry out to an extent which would have caused severe injury to a crop. There was no drainage from the pots. They were kept outside from August 27 to November 10, the rainfall being supplemented by water as necessary. From November 10 to March 14 the pots were in a heated greenhouse, and were given 2 liters of water each week.

SAMPLING AND ANALYTICAL METHODS

Samples were taken from some or all of the pots on September 12, September 24, November 10 and March 14. The samples were taken by means of a steel sampling tube, of 16.5 mm. internal diameter, driven to the bottom of the pot. About five tubes full were taken at each sampling, and the holes were not closed. The samples taken September 12 were not dried, but were mixed as well as possible and portions for analysis weighed out at once. Subsequent samplings were air-dried as rapidly as possible and ground ½ hour in a porcelain jar mill with flint pebbles.

Residual carbonate was determined in 20-gm. samples by the modified Marr method, (10) titrating the residual Ba $(OH)_2$. The usual 1 to 50 HCl was employed for the decomposition of all samples except those treated with magnesite, for which 1 to 10 HCl and boiling at atmospheric pressure were necessary. A blank on untreated soil was always run with these.

The lime-requirement determinations by the vacuum method were made in the same apparatus as was used for carbonate determinations; 20-gm. portions of the soil were boiled *in vacuo* for 2½ hours with 80 to 100 cc. of water and 2 gm. of chemically pure precipitated calcium carbonate, the carbon

dioxide evolved being estimated as in the carbonate determinations. The temperature of the boiling mixture was very near 50°C. unless otherwise stated, and in the cases of samples taken at the two later dates, the temperature was accurately controlled by adjusting the pressure within the apparatus according to the indications of a thermometer hung in the lower part of the condenser. A number of determinations made at temperatures lower and higher than 50° were made and will be discussed.

For the few nitrate determinations made, the soil was extracted with water and the phenoldisulfonic acid colorimetric method employed (11).

SAMPLES TAKEN AT THE END OF 16 DAYS

The pots containing Wooster soil with limestone finer than 100-mesh and Paulding soil with limestone were sampled September 12. These samples were not dried, but were thoroughly mixed, and the analytical work done at once. The moisture contents were determined and the results are calculated to the air-dry basis, so as to be comparable with later data. The carbonate content of the original Wooster soil, unground and at 3.7 per cent moisture content as when put in the pots, was 0.039 per cent $CaCO_3$ on the air-dry basis, or 2.0 per cent moisture. The lime requirement on the same sample was 0.214 per cent $CaCO_3$. The sample from pot 1, Wooster soil plus 0.310 per cent $CaCO_3$ in carbon limestone past 100-mesh, showed residual carbonate equivalent to 0.208 per cent $CaCO_3$ on the air-dry basis, and the lime requirement had been reduced to 0.075 per cent $CaCO_3$. Taking into account the carbonate content of the soil at the beginning, we have 0.349 less 0.208, or 0.141 per cent $CaCO_3$ disappeared. The lime-requirement data indicate a reduction in lime requirement from 0.214 to 0.075, or 0.139 per cent $CaCO_3$.

The original air-dry but unground Paulding soil showed a carbonate content of 0.010 and a lime requirement of 0.224 per cent $CaCO_3$. The sample from pot 17, Paulding soil with an addition of 0.304 per cent $CaCO_3$, supplied by limestone past 100-mesh, showed residual carbonate equal to 0.179 per cent, and residual lime requirement of 0.102 per cent $CaCO_3$. From this soil, 0.135 per cent $CaCO_3$ had disappeared, but the lime requirement had been reduced only 0.122 per cent $CaCO_3$. From the acid Wooster soil, 42 per cent and from the alkaline Paulding soil 46 per cent of the added carbonate disappeared during the period of 16 days.

SAMPLES TAKEN AT THE END OF 27 DAYS

The samples taken September 24, about a month after the experiment had been begun, included all the pots except those treated with the coarser limestone. These and subsequent samples were air-dried, and ground previous to analysis. The data are presented in table 1. In the column headed "added bases remaining as $CaCO_3$," it will be noted that the residual carbonate had decreased in the case of the Wooster soil with limestone (pot 1)

from 58 to 50 per cent of the amount added. With the Paulding soil (pot 17) the percentages were 54 and 47. The Strongsville soil (pot 16), first sampled at this time, showed only 14 per cent remaining of the same addition of limestone as was made in the case of the Paulding soil.

Calcite and magnesite (pots 6 and 7) were apparently slightly more resistant than limestone to the agencies of decomposition in the Wooster soil. Dolomite (pot 8) was considerably more resistant, as has been noted by Stewart and Wyatt (13). Precipitated calcium carbonate (pot 9) was much more easily attacked than the natural carbonates, only 39 per cent remaining, while 17 per cent of an equivalent amount of base added to pot 10 in the form of calcium oxide was present as carbonate. Magnesium oxide (pot 11) was apparently slightly less reactive than calcium oxide; less carbonate was residual, but the magnesia had not reduced the lime requirement to the same extent. Of the magnesium carbonate added to pot 12, none remained in the carbonate form. The ready decomposition of this material when mixed with soil has been noted by MacIntire (8); a greater reduction of lime requirement, however, was caused by magnesium oxide and the calcium compounds. The slag (Al), "dicalcium silicate," added to pot 13 was apparently quite active. A part of its base content seems to have changed into carbonate, since the amount of carbonate found in the soil receiving this treatment was greater than was originally present and added in the material, while the lime requirement was reduced by a figure equal to that shown where precipitated calcium carbonate was the treatment. The blast furnace slag added to pot 14 was apparently less active than any of the other materials.

The column headed "bases not found as $CaCO_3$" refers to a percentage of calcium carbonate, calculated on the basis of air-dry soil, equivalent to the bases added in any form but not residual as carbonates. In the case of carbonated materials, this figure would represent the material which has reacted with soil constituents, but with materials other than carbonates it could possibly represent material lying inert and unchanged, as well as that which has been acted upon. The column headed "$CaCO_3$ requirement reduction" contains the percentages of calcium carbonate, referred to the air-dry soils, corresponding to the difference between the lime-requirement data for the untreated soil and that of each differently treated soil. These figures for lime-requirement reduction should correspond to the differences between the bases added and the carbonate residual in the cases of those pots treated with carbonate materials, provided that there is no base consumed by biological processes such as nitrification or by reaction with soil constituents which do not exhibit their full absorptive capacity during the lime-requirement determination.

In the cases of all the pots of Wooster soil treated with natural carbonate and sampled at this time, the correspondence is quite close, and would indicate that the vacuum lime-requirement method affords indications which are quite satisfactory for purposes of comparison, at least. The Strongsville and

Paulding soils treated with limestone exhibit considerable discrepancies in this respect, however, as likewise do those pots of Wooster soil treated with the more active precipitated carbonates or oxides.

SAMPLES TAKEN AT THE END OF 75 AND 199 DAYS

The samples taken November 10 and March 14 will be discussed together. The natural carbonates have been further decomposed, and their relative resistance to decomposition does not differ materially from that indicated at the earlier sampling. The precipitated calcium carbonate has undergone further decomposition, although at the last sampling more carbonate was found in the soil from the pot treated with this material than was found at the earlier date, a circumstance difficult to account for. The second sampling indicates a slight increase in carbonate content for pot 10, treated with calcium oxide, but at the third sampling it is at the same figure as at first. The increase in carbonate content noted at the second sampling might be due to the carbonation of calcium oxide previously unaffected, although the findings of MacIntire are rather against this explanation. This investigator (9) concludes that if conditions are at all favorable to carbonation or reaction, caustic or hydrated lime does not long persist in the soil. A statement to the same effect has been attributed to Hager (5), although the writer has not seen the original article. The magnesium-oxide and carbonate treatments both appear to have resulted in a later formation of carbonate, although this is inconsiderable in the case of the magnesium carbonate.

The three soils treated with limestone past 100-mesh show in these last samples about the same relative amounts of residual carbonate as were found in the earlier samples. It seems rather remarkable that at the end of six months limestone should survive nearly as well in a soil acid to litmus paper as in one alkaline to that test, while in the acid Strongsville soil the limestone has practically disappeared at the last sampling.

The four pots of Wooster soil treated with limestone coarser than 100-mesh were sampled at the end of 75 and 199 days only. The results for carbonate content indicate that the distribution of the coarser particles of limestone through the soil in the pots was very imperfect. The indications of the lime-requirement determinations seem more reliable, although some of these are not entirely consistent. At any rate, these coarser materials are indicated to be much less active than the same stone when more finely divided, which agrees with the conclusions of several investigators, based upon evidence along other lines.

CHANGES IN LIME-REQUIREMENT REDUCTION

Upon reference to the data for $CaCO_3$-requirement reduction, assembled in table 1, it will be noted that the later samplings show a progressive increase in the lime requirement satisfied, in the case of the pots treated with the

TABLE 1

Changes in lime materials and reduction of lime requirement

POT		TREATMENT	28 DAYS			75 DAYS			199 DAYS		
			Added bases remaining as $CaCO_3$	Bases not found as $CaCO_3$	$CaCO_3$ requirement reduction	Added bases remaining as $CaCO_3$	Bases not found as $CaCO_3$	$CaCO_3$ requirement reduction	Added bases remaining as $CaCO_3$	Bases not found as $CaCO_3$	$CaCO_3$ requirement reduction
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Wooster	1	Limestone, past 100-mesh	50	0.156	0.164	48	0.161	0.157	38	0.192	0.176
Wooster	2	Limestone, 20- to 40-mesh				84	0.051	0.030	49	0.159	0.028
Wooster	3	Limestone, 40- to 60-mesh				108		0.049	76	0.075	0.082
Wooster	4	Limestone, 60- to 80-mesh				86	0.044	0.087	60	0.123	0.129
Wooster	5	Limestone, 80- to 100-mesh				74	0.080	0.091	61	0.120	0.110
Wooster	6	Calcite, past 100-mesh	53	0.147	0.145	47	0.163	0.157	36	0.200	0.191
Wooster	7	Magnesite, past 100-mesh	54	0.113	0.111	54	0.142	0.120	33	0.207	0.154
Wooster	8	Dolomite, past 100-mesh	73	0.084	0.097	71	0.091	0.101	55	0.140	0.119
Wooster	9	$CaCO_3$, precipitated	39	0.190	0.173	19	0.252	0.180	26	0.229	0.173
Wooster	10	CaO, past 100-mesh	17	0.257	0.207	20	0.248	0.189	17	0.257	0.176
Wooster	11	MgO, past 100-mesh	11	0.276	0.198	14	0.267	0.166	16	0.262	0.120
Wooster	12	$MgCO_3$, past 100-mesh		0.310	0.164	1	0.306	0.124	3	0.301	0.124
Wooster	13	Slag (Al), 100-mesh	25	0.231	0.173	29	0.219	0.166	18	0.253	0.174
Wooster	14	Slag (B.F.), 100-mesh	1	0.306	0.079	4	0.296	0.045	2	0.305	0.059
Wooster	15	Check									
Strongsville	16	Limestone, past 100-mesh	14	0.261	0.284	9	0.275	0.304	5	0.290	0.270
Paulding	17	Limestone, past 100-mesh	47	0.161	0.116	44	0.170	0.127	37	0.188	0.151

natural carbonates, possibly excepting the Strongsville soil. Those pots which received the artificially prepared amendments, however, show in a greater or less degree exactly the opposite effect. This suggests that nitrification has been very active in some of the pots, and accordingly the later samples from some of the pots were examined for nitrate content. The amounts of nitric nitrogen found, calculated to the equivalent percentage of calcium carbonate required for neutralization, are shown in table 2. The nitrate content of the original soils is not included, because it was found to be so small as to be quite negligible.

TABLE 2

Bases consumed in nitrification

POT	CALCIUM CARBONATE EQUIVALENT TO NITRATE	
	At the end of 75 days	At the end of 199 days
	per cent	*per cent*
1	0.014	
2	0.008	
3	0.009	0.032
4	0.009	0.046
5	0.014	0.060
6	0.012	
7		0.016
8		0.012
9	0.017	0.038
10	0.023	0.011
11		0.054
12		0.054
13	0.024	0.040
14	0.011	0.033
15	0.006	
16	0.023	0.071
17	0.038	0.033

The data in table 2 show that the chemically prepared oxide and carbonate of calcium and magnesium and precipitated calcium carbonate are quite active in promoting nitrification. The quantities of nitrate produced to be sure are insufficient to account for all the change in lime requirement observed in the cases of the magnesium compounds, but these must be considered to be minimum figures for nitrates. The repeated wetting of the soil in the pots would inevitably lead to the concentration of the nitrates in those portions of the soil most exposed to the air, and from which evaporation must take place. The presence of unfilled holes from former samplings would result in such a concentration in the soil immediately adjacent, and as later samples were always taken from a quarter of the pot not previously sampled, a fair share of the nitrate content was probably not secured. Besides, as Allen and Bonazzi (1) have shown, a simple water extraction, as was here used, is not sufficient for the quantitative determination of nitric nitrogen in soil.

MANNER OF PREPARING SAMPLE, TEMPERATURE AND LIME-REQUIREMENT INDICATIONS

A common ground for criticism of lime-requirement methods is that their indications are affected by comparatively slight variations in conditions. The procedure used in this investigation is no exception to the general rule. The following figures in table 3 will illustrate the point. The soil is the Wooster silt loam used for these pots, one sample being taken from the untreated check, pot 15, the other from pot 1, treated with limestone past 100-mesh equivalent to 0.310 per cent $CaCO_3$. The soil contained originally 0.029 per cent $CaCO_3$, making the carbonate content at the beginning of the experiment 0.339 per cent $CaCO_3$. At the end of 75 days, the time at which the samples were taken, there was found 0.178 per cent $CaCO_3$, indicating that 0.161 per cent $CaCO_3$ had reacted with soil constituents. The differences in lime requirement, as determined by a procedure uniform except in respect to the degree of heat employed, are seen to vary with the temperature at which the determination was made. At temperatures above 40°C., the difference appears to be constant at about 0.168 per cent $CaCO_3$. The amount of nitrate found indicated that at least 0.014 per cent $CaCO_3$ was consumed in its formation, more than sufficient to account for the excess of carbonate disappeared, even after considering the nitrate found in the soil from the check pot.

It will be recalled that there were considerable discrepancies between the amounts of base not appearing as carbonate and reductions in lime requirement in the case of pot 10, Wooster soil treated with calcium oxide. The lime-requirement data for soil from this pot, sampled at the end of 9 weeks, and determined at three temperatures, are included in table 3. The comparison in this case is between the sample from pot 10 and the original Wooster soil, the sample from the check, pot 15, having been exhausted. However, as may be seen from data for determinations at 33° and 50°C., the original sample and that from pot 15 give nearly the same values for lime requirement, when run at the lower temperatures. The data in table 3 demonstrate very plainly that the figures for lime requirement are too small except at the highest temperature, because carbonate determinations showed that the soil had absorbed the equivalent of 0.248 per cent $CaCO_3$, more than the indicated lime requirement at the lower temperatures. A certain amount of this base absorption was doubtless due to nitrification, the equivalent of 0.023 per cent $CaCO_3$ being found in the sample under consideration. If this nitrate content is considered, the reduction in lime requirement is found to be greater than the consumption of base, exclusive of that required for nitrate formation.

This discrepancy is not great, and might well be ascribed to analytical error, but as will shortly appear, a similar and even larger difference is noted in the cases of the Strongsville and Paulding soils. The explanation is simple and since it will direct attention to certain precautions necessary in studies of this kind, will be given in detail. As was stated, the samples from pot 10 dis-

cussed in table 3 are compared with the sample of Wooster soil taken at the beginning of the experiment and not with samples from the check, pot 15, since the supply from the latter was exhausted. There were no check pots for the Strongsville and Paulding soils, the comparisons in these cases being in all cases with the samples taken at the beginning. These samples were large, and to save time were ground in a large porcelain-jar mill with flint pebbles, while the smaller samples taken from pots were ground in a small mill of the same design. After grinding the samples, it was noticed that the larger jar was considerably more efficient than the smaller one, and the samples ground in it were finer, although the time of grinding had been the same. The effect of very fine grinding of the sample upon the indications of the Veitch lime-requirement method has been noted by Brown and Johnson (3). The effect as measured by the procedure used in this work is in the opposite direction, that is, finer grinding causes an increase in lime requirement instead of a decrease as with the Veitch method. The following will serve to illustrate the influence of fineness of sample upon the indications of the vacuum method, other conditions being practically the same. The soil is that used for these pots, the temperature about 50°C. The unground soil showed a lime requirement of 0.218 per cent $CaCO_3$, that ground by hand in an agate mortar 0.260 per cent, and that ground in the porcelain jar with flint pebbles for about an hour, 0.302 per cent.

The Strongsville soil, pot 16, treated with 0.304 per cent $CaCO_3$ in limestone past 100-mesh, shows with increases in temperature the same increasing difference in lime-requirement indications when compared with the original soil. Carbonate determinations indicate that 0.275 per cent $CaCO_3$ has been consumed, but the lime-requirement determinations at 50°C. indicate a difference of 0.304 per cent and at 100°C. a difference of 0.351 per cent $CaCO_3$. This soil has a high lime requirement and shows more plainly than the Wooster soil the effect of differences in the manner of preparation.

The Paulding soil, pot 17, treated with 0.304 per cent $CaCO_3$ in limestone past 100-mesh, exhibits differences in lime-requirement indications smaller than those shown by the other soils considered. Carbonate determinations indicated the disappearance of 0.170 per cent $CaCO_3$, and nitrification would account for at least 0.038 per cent $CaCO_3$, leaving 0.132 per cent to represent the base-satisfying lime requirement. As in the other cases, the difference between the lime-requirement indications obtained from determinations at 100°C. considerably exceeds the probable figure, while at 33°C. there is a deficiency. The excessive figures obtained at the higher temperature are probable due to the difference in the degree of fineness of the samples, while the deficiency at the lower temperature can be attributed only to an incomplete reaction.

TIME ALLOWED FOR REACTION, AND LIME-REQUIREMENT INDICATIONS

The fact that with a fixed time, an increase in temperature causes the difference in lime requirement between the treated and the untreated soils to approach the probable figure for base absorbed, suggests that with a fixed temperature, an increase in time allowed might show the same result.

The Wooster soil, sampled at the beginning of the experiment, showed a lime requirement of 0.283 per cent $CaCO_3$, the temperature being 50°C. and the time 2½ hours. At the end of the period stated, the absorption apparatus only was disconnected and replaced with one freshly filled, the apparatus

TABLE 3

$CaCO_3$-requirement indications at various temperatures

	30°C.	33°C.	40°C.	50°C.	60°C.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Pot 15, check	0.220	0.240	0.262	0.293	0.304
Pot 1, limestone	0.084	0.094	0.094	0.126	0.136
Difference	0.136	0.146	0.168	0.167	0.168

	33°C.	50°C.	100°C.
	per cent	*per cent*	*per cent*
Original Wooster soil	0.225	0.283	0.552
Pot 10, Wooster soil plus CaO	0.061	0.094	0.305
Difference	0.164	0.189	0.247
Original Strongsville soil	0.454	0.571	1.006
Pot 16, Strongsville soil plus limestone	0.220	0.267	0.655
Difference	0.234	0.304	0.351
Original Paulding soil	0.271	0.337	0.707
Pot 17, Paulding soil plus limestone	0.164	0.210	0.537
Difference	0.107	0.127	0.170

again evacuated and the experiment continued as before. At the end of the second 2½ hours, the lime requirement was found to be 0.023 per cent $CaCO_3$ additional, or 8 per cent of the original indication. A sample from pot 1, treated with calcium carbonate, showed 0.126 per cent and 0.019 per cent, respectively. With these soils, the additional time makes a scarcely noticeable difference in the final result.

Under the same conditions, the Strongsville soil showed in the first 2½ hours 0.570 per cent, in the second 3 hours, 0.075 per cent and in the third 1½ hours, 0.023 per cent $CaCO_3$ required. With the Paulding soil run at the same time, the figures were 0.337, 0.037, and 0.025 per cent $CaCO_3$ required. In each case the first indication is very nearly 85 per cent of the sum of the three. There are no figures corresponding to these for the Paulding and Strongsville soils treated with limestone, but if it is assumed that the same percentage

relation between the lime requirement in 2½ hours and that found in the longer period would exist with the limed soil, the 0.210 per cent $CaCO_3$ required in the case of the limed Paulding soil run 2½ hours would become 0.247 per cent. The difference between this and the sum of the several requirements of the unlimed soil is 0.152 per cent, or 0.020 per cent in excess of the base actually consumed less that corresponding to the nitrates found. A similar calculation with the data from the Strongsville samples indicates a difference in lime requirement of 0.354 per cent $CaCO_3$, considerably more than was actually added. These excessive indications are probably due to the difference in the degree of fineness of the samples, as has been explained. In spite of this factor, also applying to the comparison of soil from pot 10, Wooster soil, with calcium oxide equivalent to 0.310 per cent $CaCO_3$, with the original sample of Wooster soil, the assumption that the lime-requirement indication obtained in 2½ hours at 50°C. is 85 per cent of the maximum obtainable by long-continued action at that temperature, appears to afford correct indications. The difference in the calculated lime requirements is 0.222 per cent $CaCO_3$; the base absorbed less the nitrate found is equal to 0.225 per cent $CaCO_3$.

The data indicate that at the moderate temperature of 50°C. the reaction between the soils tested and calcium carbonate is sufficiently complete in 6 or 8 hours for differences to be practically quantitative. At lower temperatures a longer time would probably be required, at higher temperatures the time of reaction might be shorter. There does not appear to be any reason for the belief that any procedure of aeration with agitation would shorten the time; in the 200-cc. globular flasks used, the agitation accompanying boiling *in vacuo* is very thorough, while no more efficient procedure for the removal of CO_2 can be devised.

SOIL ACIDITY AND LIME REQUIREMENT

It has been stated that the Wooster and Strongsville soils are acid to litmus paper and the Truog test (14), while the Paulding soil is alkaline to litmus paper and the Veitch test (15) and gives only a very faint reaction for acidity by the Truog test. Water extracts of the original soils, air-dried and ground, were prepared in the proportion of 50 gm. of soil to 100 cc. of water, shaken occasionally for several hours and filtered through paper.

The clear filtrates were carefully compared as to color developed with various indicators, according to the plan of Wherry (16). It was easily seen that the extracts differed in reaction toward certain indicators, the Strongsville soil giving a strongly acid extract, the Wooster soil one distinctly acid, while that from the Paulding soil was distinctly alkaline. The pH values, as nearly as could be determined from the colors developed with the indicators, without any comparison with standard buffer mixtures, were within limits as follows: Wooster soil, pH 6.0 to 6.5, Strongsville soil, pH 5.5 to 6.0, Paulding soil, pH 7.0 to 7.5.

The Strongsville soil is most strongly acid, the reaction between it and limestone has been most rapid, and at the same time it has the highest lime requirement. These facts all agree with what might be expected, but a comparison of the Wooster and Paulding samples brings out some surprising features. Limestone disappears at nearly the same rate from the alkaline Paulding soil as from the acid Wooster soil, base consumed in nitrification being considered. The lime requirement of the Paulding soil is about the same as that of the Wooster soil. From this, it would seem to follow that the total amount of unsatisfied base-absorptive capacity has perhaps as much influence upon the rate of reaction between the soil and lime materials as has the intensity of acidity.

Soil acidity and lime requirement are not synonymous terms, as some have assumed them to be. A soil may have a considerable lime requirement, but may not be acid nor in need of liming. There are certainly active base absorbents unsatisfied in neutral soils—an observation by Winter (17) demonstrates this very plainly. And it has been shown that a soil, although giving an alkaline water extract, is still capable of decomposing calcium carbonate at ordinary temperatures. In view of these facts, it would seem that the idea that lime requirement is a measure of the soil acidity to be corrected by liming is in need of revision.

That a laboratory procedure which will give an accurate indication of the optimum application of lime to the soil under field conditions would be very desirable is an obvious fact. The number of methods which have been proposed for the purpose is evidence of the attention soil chemists have devoted to the subject. All of these methods are more or less arbitrary and empirical. This is not written in a spirit of criticism; any analytical procedure applied to soil, the final criterion of its value being the behavior of crops upon that soil, cannot well be otherwise in its earlier stages. From a consideration of the numerous and varied factors which govern the utilization of basic materials in the soil and the loss of bases therefrom, it would seem that no laboratory method can reasonably be expected to give more than a very roughly approximate indication of the most profitable amount of lime to apply.

It is the writer's opinion that the perfect lime-requirement method, or perhaps a better expression would be, the perfect method for the determination of base absorption, will not find its greatest usefulness in the field mentioned. Rather, it will be a measure of changes in the soil's capacity for absorption of bases, with particular reference to calcium, as the result of liming, cropping, etc. It will scarcely be expected to be any more of a guide for field treatment than the determination of total calcium might be, yet by its means, comparatively slight changes in the relations between added bases and the soil may be followed. It should be quantitative in the sense that differences are indicated with great precision, although perhaps not measuring the total capacity of the soil for reaction with bases.

The results obtained in the present investigation appear to indicate that the reaction between soil and precipitated calcium carbonate can be made quantitative to a sufficient degree to serve as the basis for a satisfactory lime-requirement method. In order to attain this end, however, it will be necessary to devote great care to the preparation of samples and all details of the actual determination must be as uniform as possible. The soil should be finely ground; a porcelain-jar mill with flint pebbles is a satisfactory means for grinding, and it would probably not be too extreme a precaution to use the same mill and pebbles for all samples to be compared, and to charge it with the same weight of soil, as well as to have the speed and time of grinding equal in all cases. Finally, it is emphasized that the indications obtained have their chief value from a comparative standpoint, and it does not matter what the absolute figures are, if differences between samples of the same soil differently treated are indicated with accuracy. Hence, any of the methods of procedure suggested, such as aspiration with agitation at room temperature or boiling at a definite temperature *in vacuo* or at atmospheric pressure, ought to be equally satisfactory, provided only that such procedure is continued for a sufficient length of time for the reaction between the soil and calcium carbonate to afford a practically quantitative indication of differences in base-absorbent capacity.

SUMMARY

Data from an experiment in which various lime materials were mixed with soil in undrained pots, watered and kept partly outside and partly under greenhouse conditions, are presented in this paper. The conclusions formed are based upon determinations of residual carbonate, lime requirement and accumulated nitrates.

A relatively pure high-calcium limestone, calcite (Iceland spar) and magnesite were very similar in behavior. Approximately one-half of an application equivalent to 3.1 tons per acre of these materials ground to pass a 100-mesh sieve had been attacked within 4 weeks, and two-thirds within 28 weeks. The natural carbonate dolomite was found to be about 50 per cent more resistant than the other materials, while precipitated calcium carbonate was much more easily attacked by the agencies of decomposition.

Caustic lime is apparently more reactive than the precipitated carbonate, although with time the differences noted become less. Carbonate formation was found to result from an application of chemically prepared magnesium oxide, and the amounts found gradually increased with time. On the other hand, the disappearance of carbonate from an equivalent application of chemically prepared magnesium carbonate was complete within a month, but traces of carbonate were afterwards formed.

Of two samples of slag tested, "dicalcium silicate" was found to react with soil as readily as precipitated calcium carbonate, but blast-furnace slag was less reactive than any other material tested.

Coarsely ground limestone had much less effect in reducing lime requirement than the finely ground.

Limestone ground to pass a 100-mesh sieve was found to be utilized at nearly the same rate in an acid silt loam and an alkaline clay loam, but carbonate disappeared much more rapidly from an acid clay loam. The lime requirements by the vacuum method were comparable in the cases of the first two soils, but twice as great with the third.

The quantitative relations between bases not residual as carbonate and reductions in lime requirement were found to be reasonably close, a possible consumption of bases by nitrification being considered. The evidence obtained in the present investigation indicates that the interaction of soil constituents and calcium carbonate is sufficiently regular and quantitative for the indications of a lime-requirement method based upon the reaction, to be of practical utility, provided the proper precautions are observed.

The indications of the lime-requirement method employed were found to depend to a great extent upon such factors as the manner of preparation of the sample, the temperature and the time allowed for the determination.

No conclusive evidence has been obtained that heating is an undesirable feature of a perfect lime-requirement method, if we understand by that term a satisfactory method for the study of the relations between base absorbents and added bases in the soil. On the other hand, heat may be of great advantage in hastening a naturally slow reaction and may enable differences to be indicated with an exactness not otherwise attainable in a reasonable length of time.

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EFFECT OF SALT SOLUTIONS HAVING DEFINITE OSMOTIC CONCENTRATION VALUES UPON ABSORPTION BY SEEDS[1]

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INTRODUCTION

The purpose of this paper is to report a part of the data obtained from a series of experiments which were designed to determine to what extent salt solutions with definite osmotic concentration values tend to resist water absorption by seeds placed in contact with them, and whether this influence has a direct bearing upon germination. The data here presented deal only with that portion of the work which has to do with the rates of imbibition as these are influenced by the osmotic concentrations of the different solutions employed.

It is, of course, generally understood that salts in solution offer resistance to water absorption by plant roots in contact with them because of the osmotic properties of the solutions, and it appears that these same physical properties influence in a somewhat similar manner the imbibition of water by seeds. Absorption from salt solutions by seeds has been studied by a number of investigators under different conditions and the results obtained are somewhat conflicting. No attempt can here be made to review the literature on imbibition by seeds and its relation to germination, but a few of the more definite contributions on this subject may be mentioned as having a direct bearing on the present study.

Slosson (5) working with a large number of different kinds of seeds has shown that the presence of salts in solution hinders the absorption of water. This same author points out that retardation of imbibition does not increase proportionately with the osmotic pressure of the solutions but less rapidly, and that isosmotic solutions produce practically the same effect, showing that osmotic pressure is of greater importance than the kind of salts.

In order to decide whether the absorption of water by seeds is physical or physiological, Buffum (2), investigating the effect of salt concentration on the germination of seeds of different species, came to the conclusion that the retarding effect of a salt solution on germination is in direct proportion to the osmotic pressure when the solutions are strong.

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Slosson and Buffum (6) made comparative tests between living and dead seeds in which the power of germination had been destroyed by age, heat, or exposure to formaldehyde vapor. They came to the conclusion that there is no difference between living and dead seeds with respect to the absorption of water, and that water is drawn into the seeds by purely physical forces such as surface tension and osmotic pressure.

Atkins (1) made an attempt to discover the forces which cause water and salt solutions to enter dried seeds. Seeds were immersed in water and in various salt solutions. Changes in the concentration of the salt solutions were determined by volumetric methods and the conclusion drawn is that "the rate at which distilled water is taken up is no greater than that at which salt solutions are absorbed," and that there is no difference in absorption between living and dead seeds until after germination. He states that the forces involved are capillarity and imbibition in the initial stages, and osmosis after germination.

Dachnowski (3), studying the effect of acid and alkaline solutions upon the water relation of plants, comes to the conclusion that the amount of water absorbed and retained by seeds in an acid solution is not dependent upon the concentration of the acid and is not a function of it. The addition to the solution of 0.00125 *N* HCl of a molecularly equivalent solution of any salt not reacting with the acid does not increase the quantity of water absorbed and retained by seeds of *Phaseolus*. However, a higher concentration of any salt is followed by an inhibition of the capacity for absorbing and retaining water. The conclusions reached for the results on absorption and retention of water by seeds in alkaline solutions are analogous to those for acids.

The work published by Shive (4) on the effect of salt concentration on the germination of seed in sand cultures clearly shows the retarding influence of solution concentration in a solid substratum upon water absorption by seeds. Single-salt solutions of definite osmotic concentration values were added to sand in the proportion of 100 cc. of solution to 1 kgm. of air-dry sand. Seeds of corn and beans were incubated in these cultures for periods of from 48 to 72 hours at room temperature. The author states that while germination was not prevented by the higher osmotic concentrations, it was considerably retarded, and that the retarded germination appeared to be directly related to the amounts of water imbibed by the seeds, which, in turn, is dependent upon the osmotic concentrations of the solutions.

The studies reported in the following pages, as already stated, deal simply with the absorption by seeds from salt solutions having definite osmotic concentration values and from distilled water. An effort was made also to determine the influence of time upon the rates of absorption by seeds from single-salt solutions and from a complete nutrient solution for plants.

METHODS

"Baker's analyzed" salts only were used. The salts used were $Ca(NO_3)_2$, K_2CO_3, $MgSO_4$, KH_2PO_4, NaCl, KCl, $NaNO_3$, and Shive's complete nutrient solution for plants, no. R_5C_2. Half-molecular stock solutions of each salt were prepared in sufficient quantities to last throughout the experiment. From these stock solutions the more dilute solutions were prepared ranging in osmotic concentration values from 0.001 atmosphere to 7.0 atmospheres. The osmotic concentration values of these solutions were calculated to approximate the concentrations desired and were then tested by the freezing-point method and corrected whenever necessary. In table 1 are given the kind of salts, the volume-molecular concentrations, and the osmotic concentration values of the solutions employed. The solutions having osmotic concentration values below 0.5 atmosphere were calculated but not tested for

TABLE 1

Osmotic and volume-molecular concentrations of solutions used

OSMOTIC CONCENTRATION	VOLUME-MOLECULAR CONCENTRATIONS						
	$MgSO_4$	KNO_3	$Ca(NO_3)_2$	KCl	K_2CO_3	KH_2PO_4	NaCl
atm.							
0.5	0.0116	0.0098	0.0073	0.0099	0.0070	0.0105	0.0159
1.0	0.0232	0.0196	0.0146	0.0199	0.0144	0.0210	0.0319
2.0	0.0530	0.0417	0.0318	0.0398	0.0308	0.0420	0.0682
3.0	0.0814	0.0633	0.0495	0.0609	0.0466	0.0630	0.1032
4.0	0.1131	0.0876	0.0698	0.0839	0.0649	0.0840	0.1438
5.0	0.1416	0.1102	0.0896	0.1052	0.0826	0.1050	0.1829
6.0	0.1759	0.1326	0.1074	0.1305	0.0989	0.1260	0.2188
7.0	0.2060	0.1585	0.1297	0.1486	0.1193	0.1472	0.2639

correctness by the freezing-point method. The solutions comprised in a series increased in osmotic concentration from 1.0 to 7.0 atmospheres by equal increments of 1.0 atmosphere. The concentrations of the more dilute solutions in each series were 0.001, 0.01, 0.10, and 0.50 atmosphere.

The seeds employed comprised corn (*Zea mays*), soybeans (*Soja maxima*), alfalfa (*Medicago sativa*), white lupine (*Lupinus alba*), spring wheat (*Triticum vulgare*), watermelon (*Citrullus vulgare*), Canada field peas (*Pisum sativum*), Japanese buckwheat (*Fagopyrum esculentum*), and dwarf Essex rape (*Brassica napus*). Fifty seeds of the larger kind and 100 each of alfalfa and rape were weighed and placed in screw-cap bottles each containing 100 cc. of solution. To each series was added one control in distilled water for each species of seed. The bottles were placed on a laboratory table and the seeds were allowed to soak for a period of 15 hours, after which time the liquid was poured off and the seeds quickly placed between filter papers to remove the water on the surface of the seeds. The seeds were then placed in small bottles

to prevent evaporation during the time of weighing. The weights of the seeds were recorded and the per cent of solution absorbed and the relative absorption quantities were calculated, the control in distilled water being used as a unit.

EXPERIMENTAL WORK

I. The influence of osmotic concentration upon absorption by seeds

The influence of salt solutions of various osmotic concentrations is shown by the data recorded in table 5, which gives the osmotic concentration values of the solutions in atmospheres and the absorption amounts per gram of dry seed relative to the amount absorbed from distilled water taken as unity. The actual amount of absorption per gram of dry seed from distilled water is given in parenthesis, so that the corresponding amount of absorption from any

TABLE 2

Variation in the absorbing power of corn of the same variety grown under the same conditions but from ears of different plants; seeds soaked for a period of 15 hours in solutions of $Ca(NO_3)_2$

CONCENTRATION	ABSORPTION	
	Seeds from ears of plant 1	Seeds from ears of plant 2
atm.	*per cent*	*per cent*
7	31.2	28.0
6	31.1	27.6
5	31.5	29.4
4	32.0	29.0
3	31.8	29.8
2	32.6	30.2
1	32.1	30.0
0.5	34.7	32.4
0.10	35.2	32.7
0.01	34.8	32.4
0.001	35.3	32.7
0.00	35.6	32.9

solution may be obtained by multiplying the relative amount by the actual amount absorbed from distilled water as given in parenthesis in the same column. All experiments were made in duplicate if not otherwise stated. The data, therefore, represent the values obtained by averaging the corresponding data from the duplicate series. This was deemed necessary after the observation, made in the course of the study, that seeds of the same kind do not always absorb the same quantities of liquid per gram of dry seed, even though the osmotic concentration, temperature, and length of time exposed are the same. A striking example of this is given in table 2, which presents the data obtained with seeds of corn of the same variety and grown to maturity in the same field under approximately the same conditions, but taken from

ears of two different plants. The seeds were immersed in solutions of calcium nitrate for 15 hours, 50 seeds in each solution. Absorption by the seeds from the different plants, in general, followed the same order with respect to solution concentration, but the per cent of absorption throughout was higher for the seeds from one plant than it was for the seeds from the other, this difference approximating 2.5 per cent as an average for this series. In order to eliminate this factor of individual differences in absorption as far as possible, large quantities of the seeds of each species were thoroughly mixed before selections were made. The seeds to be used were then carefully selected from these lots for uniformity of size, shape, and general appearance. Only healthy appearing seeds were used. The seeds having a waxy or oily covering, like rape, were stirred in the solutions at first to prevent floating.

The influence of single-salt solutions in concentrations of 0.001 to 7.0 atmospheres upon the absorption was studied and the data are recorded in table 3. The amount of absorption from solutions by seeds of the different species varies considerably. The same is true for absorption in distilled water, but for seeds of the same species there is a marked degree of uniformity in the absorption from distilled water throughout all the series. The slight differences occurring may be attributed to the differences in temperature at which the experiments were carried out. The experiments were conducted during the period from June to November, and although the temperature in the laboratory did not change as did the temperature outside, nevertheless considerable variations in temperature were unavoidable. It was found that all the controls of like series which were carried out at approximately the same temperature showed no marked differences in the per cent of water absorbed, the same being true with respect to absorption from solutions.

The average per cent of absorption in distilled water during the 15-hour period of immersion for the different seeds was 43.4 per cent for wheat, 30.4 per cent for corn, 80.5 per cent for watermelon, 47.7 per cent for buckwheat, 90.8 per cent for Canada field peas, 139.7 per cent for white lupine, 118.7 per cent for soybeans, 56.9 per cent for rape, and 144.5 per cent for alfalfa. It is thus apparent that the nature of the seeds (structure, chemical and physical properties, etc.) exercises a very strong influence in determining the rate of absorption. Seeds of the leguminous type show higher absorption rates than do the seeds of other types here employed. The highest absorption rates are indicated for alfalfa, and the lowest for corn. It is to be expected also that the size of the seeds of the different species influences their rates of absorption, since small seeds such as alfalfa present a very much greater total absorbing surface per gram of seed than do the seeds of the larger types such as soybeans or white lupine. It is clear, however, from the data of table 3, that the size of the seed is by no means the predominating influence in determining the rates of absorption.

Inspection of table 3 brings out the fact that the general effect of increasing the osmotic concentration of the solutions is to decrease the absorption. This

TABLE 3

Absorption from single-salt solutions of varying osmotic concentration values relative to the absorption per gram of dry seed in distilled water taken as unity; all seeds immersed for a period of 15 hours

CONCENTRATION	WHEAT	CORN	WATER-MELON	BUCK-WHEAT	CANADA FIELD PEAS	WHITE LUPINE	SOYBEANS	RAPE	ALFALFA
Series 1, K_2CO_3									
atm.									
7.000	0.93	1.00	0.96	0.98	0.93	1.02	0.95	1.00	1.38
6.000	0.94	1.00	0.99	0.88	0.96	1.03	0.93	0.99	1.20
5.000	0.96	1.01	1.02	0.94	0.97	1.04	0.93	0.98	1.06
4.000	0.99	1.03	1.04	1.00	0.99	1.01	0.96	1.05	1.09
3.000	0.99	1.02	1.03	1.00	0.97	1.01	0.97	1.05	1.13
2.000	0.98	1.07	1.08	1.02	0.99	1.00	0.97	1.05	0.98
1.000	0.98	1.06	1.04	0.99	0.99	0.98	0.98	1.05	0.92
0.500	1.01	1.10	1.01	0.98	0.99	1.00	0.97	0.99	1.04
0.100	1.02	1.00	0.96	1.02	0.98	0.98	0.98	0.96	1.04
0.010	1.00	1.01	0.98	1.05	0.99	0.99	0.98	0.94	1.06
0.001	1.00	0.99	0.97	1.06	1.00	1.01	0.99	0.99	1.03
0.000	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	*(0.443)	(0.302)	(0.786)	(0.464)	(0.890)	(1.451)	(1.237)	(0.591)	(1.442)
Series 2, $Ca(NO_3)_2$									
7.000	0.94	0.88	0.88	0.94	0.92	0.84	0.87	0.89	0.73
6.000	0.94	0.88	0.90	0.93	0.94	0.85	0.89	0.95	0.72
5.000	0.91	0.88	0.88	0.91	0.96	0.86	0.89	0.95	0.71
4.000	0.93	0.90	0.92	0.94	0.94	0.89	0.90	0.97	0.75
3.000	1.00	0.90	0.91	1.02	0.93	0.91	0.89	0.97	0.76
2.000	1.00	0.91	0.90	0.96	0.96	0.92	0.93	0.97	0.75
1.000	1.02	0.89	0.89	0.98	0.96	0.93	0.94	0.97	0.80
0.500	1.03	0.97	0.92	1.02	1.00	0.92	0.94	0.97	0.81
0.100	1.02	0.99	0.96	1.01	1.01	0.99	0.99	0.96	0.77
0.010	1.02	0.98	0.98	1.11	1.00	1.00	1.00	0.96	0.88
0.001	0.97	0.99	1.03	1.06	1.00	1.00	1.00	0.98	0.91
0.000	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(0.455)	(0.356)	(0.798)	(0.478)	(0.977)	(1.469)	(1.242)	(0.550)	(1.560)
Series 3, $MgSO_4$									
7.000	0.79	0.78	0.83	0.89	0.95	0.81	0.87	0.91	0.74
6.000	0.85	0.82	0.85	0.93	0.95	0.85	0.87	0.95	0.76
5.000	0.85	0.87	0.86	0.95	0.95	0.89	0.90	0.95	0.84
4.000	0.85	0.86	0.90	0.94	0.97	0.91	0.94	0.97	0.81
3.000	0.88	0.88	0.90	0.98	0.97	0.92	0.93	0.97	0.91
2.000	0.88	0.88	0.90	0.97	0.98	0.97	0.94	0.99	0.87
1.000	0.92	0.91	0.92	1.05	1.01	0.98	0.94	1.01	0.90
0.500	0.92	0.95	0.93	1.00	1.03	0.96	0.95	1.00	0.91
0.100	0.94	0.95	0.94	1.09	1.02	0.99	0.97	1.06	0.98
0.010	0.97	0.98	1.02	1.08	1.01	1.02	0.99	1.02	1.00
0.001	0.96	0.99	0.97	1.05	1.02	1.01	0.99	1.03	1.05
0.000	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(0.477)	(0.445)	(0.905)	(0.453)	(0.867)	(1.340)	(1.215)	(0.580)	(1.405)

TABLE 3—*Continued*

CONCENTRATION	WHEAT	CORN	WATER-MELON	BUCK-WHEAT	CANADA FIELD PEAS	WHITE LUPINE	SOYBEANS	RAPE	ALFALFA
				Series 4, NaCl					
7.000	0.95	0.88	0.80	0.81	0.92	0.87	0.91	0.95	0.82
6.000	0.96	0.88	0.84	0.83	0.94	0.89	0.93	0.99	0.85
5.000	0.99	0.88	0.90	0.92	0.94	0.92	0.93	0.92	0.85
4.000	1.01	0.91	0.90	0.86	0.94	0.93	0.93	0.93	0.92
3.000	0.99	0.95	0.90	0.89	0.96	0.93	0.95	0.93	0.84
2.000	1.01	0.98	0.88	0.93	0.97	0.96	0.96	0.93	0.88
1.000	1.04	0.95	0.98	1.02	1.00	0.99	0.97	1.02	0.88
0.500	1.07	1.00	1.01	0.96	0.98	0.98	1.01	0.94	0.88
0.100	1.07	0.99	0.98	0.94	0.98	1.00	1.01	0.95	0.99
0.010	1.05	1.02	1.00	1.01	0.96	1.01	1.01	0.95	0.97
0.001	1.05	0.98	0.94	1.01	0.98	1.02	1.01	0.95	1.01
0.000	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(0.408)	(0.284)	(0.813)	(0.492)	(0.932)	(1.341)	(1.181)	(0.612)	(1.460)
				Series 5, KCl					
7.000	0.97	0.88	0.75	0.90	0.93	0.89	0.85	0.94	0.81
6.000	0.96	0.90	0.78	0.89	0.94	0.90	0.88	0.97	0.82
5.000	0.90	0.95	0.80	0.89	0.96	0.92	0.92	0.96	0.82
4.000	0.95	0.95	0.80	0.90	0.96	0.94	0.93	0.97	0.82
3.000	0.98	0.94	0.87	0.91	0.96	0.95	0.96	1.00	0.86
2.000	0.97	0.96	0.86	0.96	1.01	0.96	0.95	1.02	0.88
1.000	0.98	0.95	0.89	1.01	0.98	1.00	1.06	1.01	0.88
0.500	1.00	0.97	0.94	1.05	1.00	0.99	0.99	1.06	0.91
0.100	0.99	1.03	0.94	1.05	0.99	1.02	1.01	1.00	1.00
0.010	0.97	1.01	1.06	1.00	0.98	0.99	1.00	1.03	0.98
0.001	0.99	1.00	1.05	1.05	0.98	1.00	0.99	1.00	1.00
0.000	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(0.443)	(0.277)	(0.855)	(0.479)	(0.896)	(1.390)	(1.108)	(0.536)	(1.470)
				Series 6, $NaNO_3$					
7.000	0.95	0.91	0.89	0.86	0.97	0.89	0.90	1.00	0.84
6.000	0.95	0.93	0.90	0.85	0.99	0.92	0.89	1.00	0.88
5.000	0.92	0.93	0.97	0.87	1.00	0.91	0.93	0.96	0.89
4.000	0.97	0.93	0.97	0.91	0.99	0.93	0.96	1.02	0.91
3.000	0.96	0.97	0.96	0.91	1.00	0.93	0.96	1.06	0.88
2.000	0.95	0.97	1.01	0.92	1.01	0.93	0.96	1.13	0.95
1.000	0.97	0.97	1.02	0.93	1.02	0.95	0.97	1.02	0.90
0.500	0.97	0.96	1.01	0.97	1.04	0.97	1.00	1.06	0.90
0.100	0.97	0.99	0.96	0.96	1.04	0.96	1.01	1.07	0.93
0.010	0.96	0.99	1.00	0.96	1.03	0.96	1.02	1.04	0.94
0.001	0.97	0.99	1.01	0.96	1.03	0.96		1.03	0.96
0.000	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(0.446)	(0.299)	(0.771)	(0.498)	(0.883)	(1.393)	(1.140)	(0.545)	(1.441)

* The actual amount of absorption per gram of dry seed in distilled water is given in parentheses in grams. The corresponding amount of absorption from any solution may be calculated by multiplying the relative weight by the actual weight absorbed in distilled water.

is true of all the different species of seeds in the solutions of all the salts used except K_2CO_3. The decrease in absorption with an increase in the osmotic concentrations of the solutions is, of course, more uniform for some species of seeds than for others, and for any one species it is more uniform in some of the solutions than in others. It is to be noted, however, that low concentrations of some of the salt solutions appear to have a stimulating effect upon the absorption of some of the species, but not at all upon others. Thus, in the series with $MgSO_4$, there is indication that absorption by seeds of watermelon, buckwheat, Canada field peas, white lupine, rape, and alfalfa is stimulated by low concentrations of this salt, but absorption by wheat, corn, and soybeans is not thus affected. This stimulating effect upon the absorption by some of the seeds is fairly general throughout all of the series.

TABLE 4

Relative absorption data, being the average for the seeds of all species separately in single-salt solutions of corresponding concentrations of each salt; condensed summary of table 3

CONCENTRATION	K_2CO_3	$Ca(NO_3)_2$	$MgSO_4$	NaCl	KCl	$NaNO_3$
atm.						
7	1.02	0.86	0.84	0.88	0.88	0.91
6	0.99	0.89	0.87	0.90	0.89	0.92
5	0.99	0.88	0.89	0.92	0.90	0.93
4	1.02	0.90	0.91	0.93	0.91	0.95
3	1.02	0.92	0.93	0.93	0.94	0.96
2	1.02	0.92	0.93	0.94	0.95	0.89
1	1.00	0.93	0.96	0.98	0.97	0.97
0.5	1.01	0.95	0.96	0.98	0.99	0.99
0.1	0.99	0.97	0.99	0.99	1.00	0.99
0.01	1.00	0.99	1.01	1.00	1.00	0.99
0.001	1.00	0.99	1.01	0.99	1.01	0.99
0.00	1.00	1.00	1.00	1.00	1.00	1.00

Absorption in the K_2CO_3 series was very irregular. The solutions of this salt, being strongly alkaline in reaction, had the effect of softening not only the seed-coats but also the seeds themselves, the softening effect increasing proportionally with the concentration of the solutions. This chemical attack upon the seed-coats and seeds had a marked effect upon the rate of absorption, as is clearly indicated in the case of alfalfa. Absorption by this seed rapidly increased with an increase in concentration of the solution, the greatest rate of absorption occurring with the highest concentration. Other seeds, such as the white lupine and rape, were somewhat similarly affected, while wheat and Canada field peas appeared to be but very slightly influenced. The solutions of K_2CO_3 further exhibited a strong tendency to extract coloring matter from the seed-coats, a property which appeared to be entirely lacking in the solutions of all the other salts used. The seeds having colored pigments in their coats thus imparted to the solutions of this salt characteristic colorations, the degree of color depending upon the concentration of the solutions.

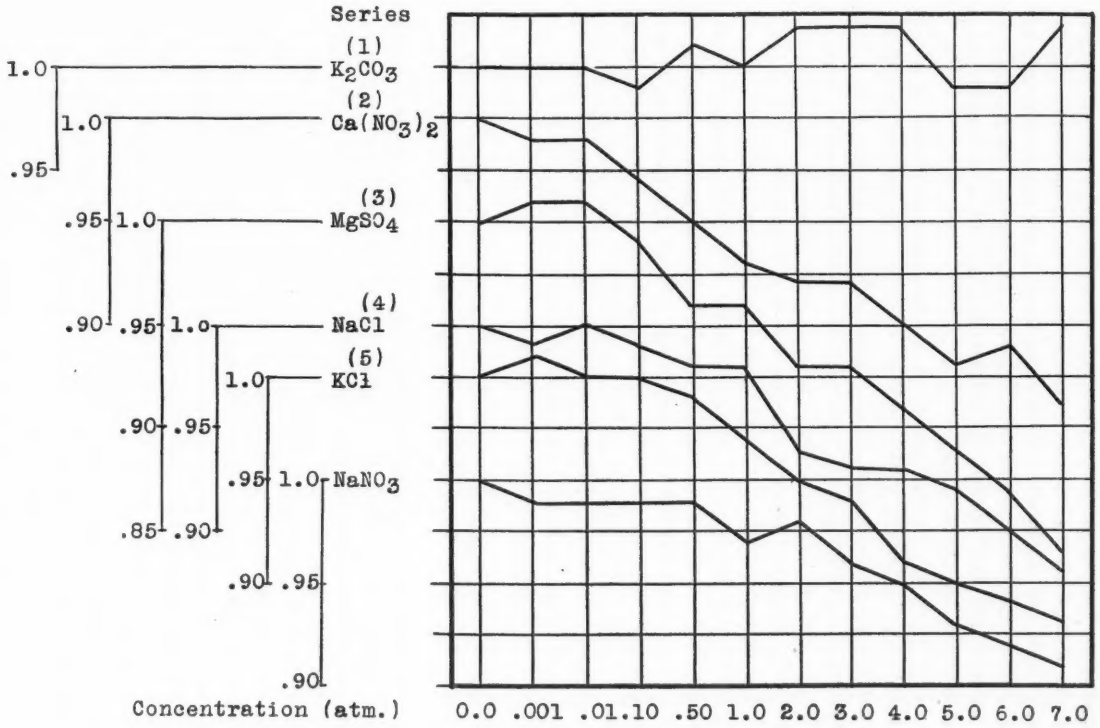

FIG. 1. GRAPHS SHOWING THE INFLUENCE OF OSMOTIC CONCENTRATIONS OF SINGLE-SALT SOLUTIONS UPON RELATIVE ABSORPTION RATES

The general effect of salt concentration upon absorption is still further brought out by the data of table 4, which shows the average relative absorption quantities of the seeds of all species separately in corresponding concentrations of each single salt employed. These data, being a condensed summary of table 3, are represented graphically in figure 1. The average relative absorption amounts are here plotted as ordinates against osmotic concentration values in atmospheres as abscissas.

It will be observed that the graphs of all the series except that of series 1 (K_2CO_3) agree in showing a fairly uniform decrease in absorption quantities with an increase in the osmotic concentration values of the solutions. The stimulating effect of low osmotic concentrations upon absorption as brought out by the detailed data in table 3, is not clearly shown in these graphs, although the graphs of series 3 and series 5 do indicate slightly increased relative absorption quantities for some of the lower concentrations.

The chemical action upon the seed-coats and seeds of the solutions used in series 1 (K_2CO_3) and its consequent effect upon absorption from these solutions, as pointed out in connection with the data of table 3, is very clearly shown by the graph representing this series. This graph does not show the general downward slope to the right as do the graphs of the other series, while the average relative absorption from five different concentrations of the salt used in this series, including the highest concentration, is actually greater than that from distilled water (0.0 concentration).

II. Influence of time upon the absorption by seeds from solutions varying in osmotic concentration values

The conclusion drawn by Atkins (1) that "the rate at which distilled water is taken up is no greater than that at which salt solutions are absorbed" may hold for solutions with low osmotic concentrations; but with the higher concentrations used in the present study the absorption rates were decreased to a marked degree, as is clearly shown by the results of the preceding experiments. In order to determine whether the rates of absorption by the seeds would tend to equalize in solutions of different concentrations with continued mmersion, experiments were carried out in which the periods of soaking varied from 15 to 149 hours. For convenience, only the larger seeds, corn, watermelon and beans, were employed. Single-salt solutions of $Ca(NO_3)_2$, KCl, KNO_3, KH_2PO_4, and K_2CO_3 were used. Tests were also made with Shive's three-salt solution, number R_3C_2. The osmotic concentration values of the solutions in each series varied from 0.5 to 7.0 atmospheres and each series included controls with distilled water.

The seeds were soaked for a period of 15 hours in the manner previously described. At the end of this period each lot of seeds was removed from the solution and placed between double layers of filter paper saturated with the solution in question, and the whole was then placed in a moist chamber. Care was taken to keep the layers of filter paper enclosing each lot of seeds con-

stantly saturated with the proper solution during the entire period of incubation. Weights were recorded and the absorption amounts per gram of dry substance were calculated as before. The results obtained are presented in table 5.

TABLE 5

Influence of length of time upon the relative absorption of seeds in single-salt solutions of varying osmotic concentration values; absorption from distilled water per gram of dry seed considered as 1.00

CONCENTRATION	KCl								
	Beans			Corn			Watermelon		
	29 hrs.	48 hrs.	72 hrs.	15 hrs.	68 hrs.		15 hrs.	71 hrs.	96 hrs.
atm.									
7.0	0.87	0.86	0.83	0.88	0.64		0.75	0.56	0.70
6.0	0.91	1.00	0.87	0.90	0.70		0.78	0.70	0.70
5.0	0.94	1.10	0.89	0.95	0.77		0.80	0.77	0.71
4.0	0.97	0.97	0.93	0.95	0.83		0.80	0.75	0.75
3.0	0.92	0.94	1.00	0.94	0.86		0.87	0.79	0.82
2.0	0.95	0.93	1.01	0.97	0.92		0.86	0.88	0.95
1.0	0.96	1.06	1.02	0.95	0.93		0.89	0.99	1.08
0.5	1.03	1.27	1.07	0.97	0.98		0.94	0.97	1.14
0.0	1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00
	(1.140)	(1.217)	(1.432)	(0.277)	(0.506)		(0.855)	(0.909)	(1.022)
	$Ca(NO_3)_2$								
	30 hrs.	48 hrs.	51 hrs.	15 hrs.	70 hrs.		15 hrs.	120 hrs.	
7.0	0.89	0.77	0.74	0.94	0.45		0.88	0.58	
6.0	0.91	0.80	0.79	0.94	0.47		0.90	0.75	
5.0	0.92	0.84	0.78	0.91	0.54		0.88	0.77	
4.0	0.93	0.86	0.80	0.93	0.60		0.92	0.64	
3.0	0.95	0.88	1.01	1.00	0.66		0.91	0.67	
2.0	0.96	0.92	0.89	1.00	0.85		0.90	0.90	
1.0	0.99	1.04	1.02	1.02	1.02		0.89	0.79	
0.5	0.99		1.15	1.03	0.99		0.92	1.00	
0.0	1.00	1.00	1.00	1.00	1.00		1.00	1.00	
	(0.932)	(1.124)	(1.176)	(0.455)	(0.818)		(0.798)	(0.989)	
	KH_2PO_4								
	43 hrs.	68 hrs.		47 hrs.	69 hrs.		15 hrs.	119 hrs.	149 hrs.
7.0	0.86	0.91		0.73	0.65		0.82	0.75	0.49
6.0	0.96	0.87		0.77	0.70		0.83	0.86	0.52
5.0	0.91	0.90		0.79	0.73		0.86	0.81	0.51
4.0	0.90	0.93			0.73		0.86	1.16	0.79
3.0	0.93	0.93		0.89	0.89		0.87	1.29	0.92
2.0	0.96	0.96		0.89	0.96		0.92	1.45	0.98
1.0	1.04	0.92		1.03	0.89		0.94	1.80	1.05
0.5	1.02	0.93		1.02	1.11		1.02	1.18	0.94
0.0	1.00	1.00		1.00	1.00		1.00	1.00	1.00
	(1.191)	(1.291)		(0.459)	(0.628)		(0.648)	(0.907)	(1.439)

TABLE 5—*Continued*

CONCENTRATION	KNO_3								
	Beans			Corn			Watermelon		
	42 hrs.	72 hrs.		15 hrs.	49 hrs.	52 hrs.			
atm									
7.0	0.86	0.80		0.90	0.83	0.69			
6.0	0.86	0.83		0.92	0.76	0.77			
5.0	0.94	0.81		0.92	0.85	0.83			
4.0	0.90	0.91		0.92	0.80	0.89			
3.0	0.94	0.92		0.96	0.85	0.95			
2.0	0.98	1.00		0.96	0.91	1.00			
1.0	1.02	1.01		0.96	0.94	1.03			
0.5	1.01	1.00		0.96	1.02	1.01			
0.0	1.00	1.00		1.00	1.00	1.00			
	(0.994)	(1.228)		(0.342)	(0.468)	(0.602)			
	$MgSO_4$								
	15 hrs.	48 hrs.		15 hrs.	50 hrs.	68 hrs.			
7.0	0.86	0.89		0.78	0.67	0.47			
6.0	0.86	0.90		0.82		0.47			
5.0	0.89	0.90		0.87	0.69	0.50			
4.0	0.94	0.96		0.86	0.70	0.53			
3.0	0.92	0.99		0.88	0.75	0.61			
2.0	0.93	1.00		0.88	0.60	0.63			
1.0	0.93	1.00		0.91	0.79	0.79			
0.5	0.98	1.01		0.96	0.87	0.88			
0.0	1.00	1.00		1.00	1.00	1.00			
	(0.724)	(0.955)		(0.445)	(0.503)	(0.806)			
	K_2CO_3								
	50 hrs.	68 hrs.		15 hrs.	55 hrs.	72 hrs.			
7.0	0.86	0.93		1.00	0.70	0.52			
6.0	0.92	0.90		1.00	0.70	0.54			
5.0	0.93	0.91		1.01	0.74	0.59			
4.0	0.99	0.84		1.03		0.61			
3.0	0.97	0.92		1.02	0.86	0.69			
2.0	0.93	0.93		1.07	0.91	0.74			
1.0	0.99	1.01		1.06	0.98	0.89			
0.5	1.00	0.96		1.10	0.93	0.83			
0.0	1.00	1.00		1.00	1.00	1.00			
	(1.095)	(1.360)		(0.302)	(0.507)	(0.679)			

TABLE 5—*Continued*

CONCENTRATION	Shive's three-salt solution R_5C_2							
	Beans		Corn			Watermelon		
	15 hrs.	48 hrs.	47 hrs.	67 hrs.		24 hrs.	113 hrs.	
atm.								
7.0	0.92	0.87	0.84	0.73		0.87	0.65	
6.0	0.94	0.93	0.85	0.74		0.91	0.61	
5.0	0.97	0.94	0.88	0.80		0.93	0.72	
4.0	0.97	0.94	0.86	0.82		0.93	0.80	
3.0	1.01	0.91	0.88	0.84		0.97	0.70	
2.0	1.00	0.92	0.91	0.96		0.98	0.80	
1.0	1.02	0.94	0.94	0.91		0.98	0.86	
0.5	1.00	0.99	1.03	1.22		0.96	0.77	
0.0	1.00	1.00	1.00	1.00		1.00	1.00	
	(1.036)	(1.188)	(0.403)	(0.602)		(0.668)	(1.147)	

It should be stated here that the seeds soaked for the longer periods of time made considerable growth, and this perhaps may have had some slight influence upon the results obtained. It is of interest to point out that by far the greatest amounts of growth were made from the seeds in the solutions of lower concentrations, as indicated by measurements of the lengths of roots and shoots produced. Germination and growth rates were increasingly retarded with an increase in the osmotic concentrations of the solutions. Special attention has been given to this phase of the problem, which will be treated in detail in another study.

The data of table 5 show the same general relations between absorption rates and the osmotic concentration values of the solutions used as do the data of table 3. With longer incubation periods, however, the relative absorption amounts show more pronounced differences in passing from the lower to the higher concentrations, than they do with the shorter periods. This is true, in general, throughout all the series, including the series in which mixed solutions (Shive's three-salt solution R_5C_2) were employed. This influence of the time element upon absorption rates is more clearly brought out by the data of table 6 and by the graphs of figure 2. The data of table 6, which is a summary of table 5, represent the average relative absorption amounts for the seeds of each species separately, for the short and for the long absorption periods in all the solutions of corresponding concentrations. The average time of the short and of the long absorption periods is given at the head of the respective columns in the table. Each pair of graphs in figure 2 represents the average relative absorption data for a single species, the continuous line and the dotted line indicating the data for the short and for the long absorption periods, respectively. The absorption amounts as ordinates are here plotted against the osmotic concentration values as abscissas.

It will be observed that the graphs of each pair show considerable divergence, the lower one of each pair representing the average relative absorption amounts for the long-time periods. This divergence of the graphs is clear evidence that the rates of absorption by the seeds from solutions of different osmotic concentrations do not tend to equalize when the seeds are exposed to the solutions for longer periods of time. On the contrary, the graphs bring out the fact that the retarding influence of the solutions of higher concentrations upon the rates of absorption is more pronounced for the longer absorption periods than it is for the shorter.

From the preceding experiments it is clear that the general effect of increasing the osmotic concentration of the solutions is to retard the rate of imbibition by seeds in contact with them irrespective of the kind or nature of the salts

TABLE 6

Relative absorption data, being the average for seeds of each species in all the solutions of corresponding concentration but for time periods of different average lengths, condensed summary of table 5

CONCENTRATION	BEANS		CORN		WATERMELON	
	Average of short-time periods, 32.0 hrs.	Average of long-time periods, 58.7 hrs.	Average of short-time periods, 24.1 hrs.	Average of long-time periods, 66.1 hrs.	Average of short-time periods, 17.2 hrs.	Average of long-time periods, 119.5 hrs.
atm.						
7.0	0.88	0.85	0.87	0.59	0.83	0.60
6.0	0.92	0.87	0.89	0.63	0.85	0.64
5.0	0.93	0.88	0.90	0.65	0.87	0.68
4.0	0.94	0.90	0.93	0.72	0.88	0.74
3.0	0.95	0.95	0.94	0.79	0.90	0.78
2.0	0.96	0.96	0.95	0.97	0.91	0.91
1.0	0.99	0.99	0.98	0.92	0.93	0.93
0.5	1.00	1.02	1.01	0.99	0.96	0.96
0.0	1.00	1.00	1.00	1.00	1.00	1.00

employed, provided they do not react in solution with the seed substance to modify in a chemical way the imbibing properties of the seed as do the solutions of potassium carbonate. Dachnowski (3) has pointed out that while seeds show a greater water content in solutions of acids and alkalies than they do in distilled water, an optimal point is reached beyond which a further concentration of the acid or alkali is not followed by a greater water content but by a lesser one. The author states that no conception of colloidal swelling alone can be brought into harmony with the maximal values of water retained nor with the chemical changes which actually take place through which seeds are progressively altered. It thus appears that the retarding influence upon the rate of imbibition by the salts here employed is accomplished through the osmotic resistance offered to water entrance into the seeds. This is in entire accord with the conclusion of Slosson (5) and Buffum (2).

The average absorption rates from the salt solutions show an approximately linear relation to the osmotic concentrations of the solutions, decreasing with an increase in the concentration except in dilute solutions of some of the salts which appear to have a stimulating effect upon the rate of imbibition of the

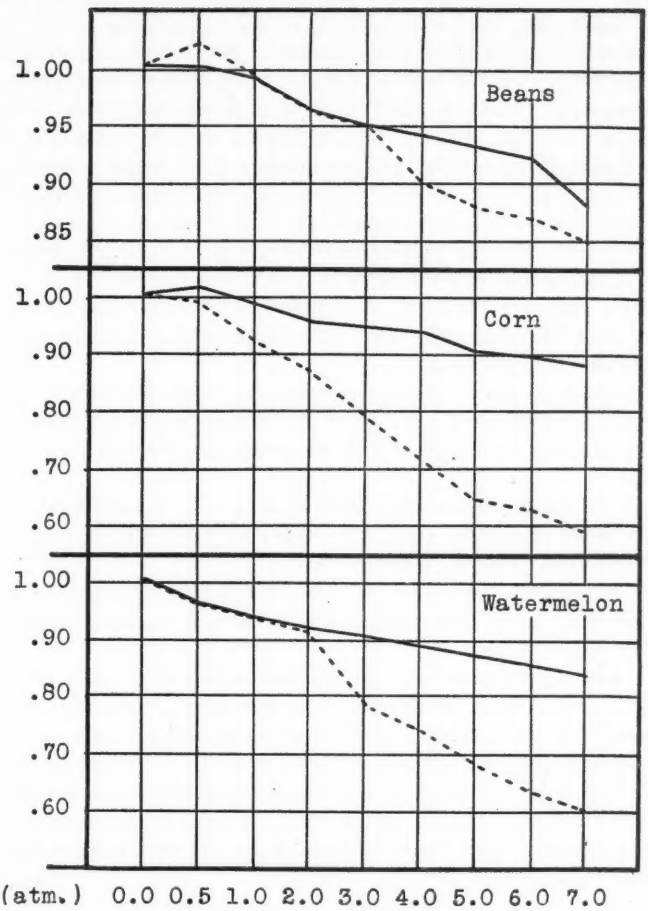

FIG. 2. GRAPHS SHOWING THE INFLUENCE OF TIME UPON RELATIVE ABSORPTION QUANTITIES IN SOLUTIONS OF VARYING OSMOTIC CONCENTRATIONS

Upper graph of each pair represents average absorption for short-time periods, lower graph for long-time periods.

seeds of certain species but not upon the rate of others. The physiological significance of these accelerated absorption rates in low concentrations of the salts employed has not been determined nor is it entirely clear from the data at hand whether any such significance can be attached to them.

SUMMARY

A study was made of the influence of salt solutions of varying osmotic concentrations upon the absorption by seeds of different species in contact with them. Single-salt solutions of $Ca(NO_3)_2$, K_2CO_3, $MgSO_4$, KH_2PO_4, NaCl, KCl, and $NaNO_3$ were employed. A complete nutrient solution for plants (Shive's three-salt solution no. R_5C_2) also was used. The osmotic concentration values of the solutions of each salt were made to vary from 0.001 atmosphere to 7.0 atmospheres. Seeds of wheat, corn, watermelon, buckwheat, Canada field peas, white lupine, soybeans, rape, and alfalfa were immersed in the solutions for definite periods of time and the amounts of absorption from the solutions and from distilled water were determined. The influence of time upon the absorption from the solutions and from distilled water also was studied.

The results of the experiments may be summarized briefly as follows:

1. There is a marked difference in the absorbing power of seeds of different species. Seeds of the leguminous type show higher rates of absorption than do seeds of the other types used. The highest absorption rates are indicated for alfalfa, the lowest for corn.

2. The rates of absorption are progressively retarded with an increase in the osmotic concentration values of the solutions which do not react with the seed substance to modify in a chemical way the imbibing properties of the seeds.

3. Average absorption rates show an approximately linear relation to the osmotic concentration values of the solutions, decreasing with an increase in concentration except in dilute solutions.

4. Experimental evidence points to the conclusion that retardation of the absorption rates is accomplished through osmotic resistance offered to water entrance into the seed.

5. Low osmotic concentrations appear to have a stimulating influence upon the absorption rates of the seeds of some species but not upon the rates of others. The physiological significance of these accelerated absorption rates in solutions of low osmotic concentration values has not been determined.

6. Increasing the length of the time period during which the seeds are in contact with the solutions has the effect of increasing the differences between the quantities absorbed from solutions of low and from those of high osmotic concentration values.

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A COMPARISON OF THE TECHNIC RECOMMENDED BY VARIOUS AUTHORS FOR QUANTITATIVE BACTERIOLOGICAL ANALYSIS OF SOIL

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A quantitative bacteriological analysis of soil for total numbers of microorganisms has but a comparatively small significance as compared with that for the estimation of numbers in one or more physiological groups. Remy (25) and Hiltner and Störmer (18) recognized this as early as 1902–1903. The principles underlying the technic used for the enumeration of total numbers of microörganisms, however, lend themselves as well to the estimation of numbers in one or more physiological groups, the only difference being in the media employed (18, 28).

Certain details of the technic recommended by various authors for analyzing soil bacteriologically are widely divergent, so much so in fact that the data obtained from one soil analyzed simultaneously by the various methods would be wholly incomparable. Up to the present time each investigator has been a law unto himself in so far as the technic used in quantitative bacteriological soil analysis is concerned. For example, King and Doryland (20) used the volumetric method, i.e., adding 19 cc. of diluting liquid to 1 cc. of soil, stating that "This method is undoubtedly inaccurate, as it is difficult to measure such substances as soil in terms of cubic centimeters. However, for the purpose of securing comparative results, as in the present work, either method may be used. . . . . The final results would appear to be correct so long as one or the other method of collecting samples is followed with uniformity throughout the work." (By the method of "collecting" samples is meant the volumetric or gravimetric methods.)

Kellerman and Allen (19) state "Though it is recognized that the methods suggested by different investigators are not adequate for accurate quantitative investigations of bacterial functions and conditions in various soils, the methods which at this time have been found most convenient and suitable for the investigations under discussion are briefly reviewed."

Bacteriologists who have experimented with soils to a considerable extent appreciate the fact that even when any one method or technic is employed, quantitative results are not infrequently very erratic. Chester (4) realized that unless one method is used for quantitative bacteriological soil analysis, comparative results could not be obtained. To quote, "In analyses of other soils, these same conditions should be duplicated, otherwise reliable comparative results cannot be expected."

A comparison has been made of the quantitative soil bacteriological methods recommended by the authors of soil bacteriology and other laboratory manuals, and also of comparatively recent articles dealing with or using quantitative methods for bacteriological soil analysis. This brings to light the information that, at the present time, no one method is used. The several points about which the various authors differ are as follows:

(a) The amount of soil used; (b) the proportion of the soil to the diluting liquid in the first dilution; (c) the size of the second dilution; (d) the method of making the first dilution; (e) the technic for transferring dilutions containing visible amounts of soil; (f) the basis for the estimation of numbers (total or otherwise).

These points of difference will be taken up in order.

Amounts of soil varying from 0.25 gm. to 100 gm. are used in the first dilution.

TABLE 1

Variations in amounts of soil used in first dilutions

NUMBER OF RECOMMENDATIONS	FIRST DILUTION		AUTHORS
	Soil used	Approximate amount of diluting liquid	
	gm.	*cc.*	
1	0.25	50	Ellis (8)
1	0.5	100	Chester (4), Conn (6)
7	1.0	99	Gainey and Gibbs (12)
		100	Savage (26), Schneider (27)
		300	Kellerman and Allen (19)
		1,000	Frost (11), Hastings and Wright (15), Löhnis (21)
2	10.0	100	Eyre (9), Heinemann (16)
3	20.0	200	Fred (10), Giltner (13), Hills (17)
1	50.0	200	Noyes and Voigt (24)
1		500	Remy (25)
4	100.0	200	Brown (2), Burgess (3), Greaves and Carter (14), Whiting (31)
1	Not stated		Waksman (29)
1	1 cc.	19	King and Doryland (20)

The proportion of soil to diluting liquid employed in the first and second dilutions varies as shown in table 2.

No attention was paid by the author of this paper as to how further dilutions were made, as the first two are deemed the most important from the standpoint of the technic in making plates or other cultures.

With four exceptions, the first dilution, almost as if by common consent, is made by adding the weighed soil to the measured amount of water. Chester, Gainey and Gibbs, Heinemann, and King and Doryland are the only ones who consider the weight or volume of the soil in making the first dilution. Gainey and Gibbs prepare the first dilution by adding 1 or 2 gm. of soil to 99 or 98

cc. of dilution liquid as desired. King and Doryland add 1 cc. of soil to 19 cc. of diluting liquid. Chester, and Heinemann, on the other hand, are the only ones who employ the technic recognized in pharmaceutical practice. They prepare the first dilution by triturating the soil (0.5 and 10 gm., respectively)

TABLE 2

Variations in proportion of soil to diluting liquid in first and second dilutions

NUMBER OF RECOMMENDATIONS	FIRST DILUTION	SECOND DILUTION	MADE BY PLACING	AUTHORS
3	1:2	1:100	2 cc. + 98 cc.	Whiting
		1:200	1 cc. + 99 cc.	Burgess, Greaves and Carter
		1:2,000	Not stated	Brown
1	1:4	1:40	10 cc. + 90 cc.	Noyes and Voigt
7	1:10	1:100	1 cc. + 9 cc.	Eyre
			10 cc. + 90 cc.	Fred, Giltner
		1:1,000	1 cc. + 99 cc.	Heinemann
		1:2,000	1 cc. + 199 cc.	Waksman
		Not stated	Not stated	Remy
1	1:20	1:2,000	Plates loopful using platinum loop of 0.001 cc. capacity	King and Doryland
1	1:50	*		Gainey and Gibbs
3	1:100	*		Gainey and Gibbs
		1:1,000 or less	1 cc. or less + 9 cc. or more	Schneider
		1:10,000	1 cc. + 99 cc.	Savage
3	1:200	Not stated	Not stated	Conn
		1:800	Plates 0.25 cc.	Ellis
		1:20,000	1 cc. + *q. s.* = 100 cc.	Chester
1	1:300	None	Plates 1 cc. of first dilution	Kellerman and Allen
3	1:1,000	1:2,000	Plates 0.5 cc.	Frost
		1:10,000	1 cc. + 9 cc.	Hastings and Wright, Löhnis

* Dilutions made in the "ordinary way."

in a mortar with a small amount of the diluting liquid, then add a sufficient quantity of the diluting liquid to make a total of 100 cc. With the exception of Schneider who triturates 1 gm. with 100 cc. of diluting liquid, all other authors reviewed here mix the soil with the diluting liquid merely by shaking for a specified time or number of times.

The size of the second dilution is important also. As with the first, an attempt should be made to obtain as representative a sample as possible for transfer into the second dilution flask. This is not possible when much less than one-tenth of the soil in the first dilution is transferred to the second dilution flask. By consulting tables 1 and 2 it will be seen that Fred and Giltner are the only ones who recommend the ideal (according to the writer's idea) first and second dilutions (1:10 and 1:100, respectively). In fact, these dilutions should be credited to Fred alone, as the writer used Fred's dilutions, considering them as ideal, in writing up this experiment for Giltner's Manual. Schneider's method which recommends 1:100 as the first dilution and 1:1000 as the second, is next in desirability. Noyes and Voigt state that the second and succeeding dilutions should differ from one another by 10. They also show by experiment that considerable error is eliminated when 10 cc. of a dilution is added to 90 cc. of the diluting liquid for making the succeeding dilution rather than by adding 1 cc. to 9 cc., proving that the results of working the two ways are not the same. The writer agrees almost wholly with Noyes and Voigt, with the exception that when a dilution becomes high enough to have eliminated all visible soil particles there seems to be no reason why succeeding dilutions should not be made by transferring 1-cc. portions to 99 cc. of diluting liquid. This would not prevent making plates or other cultures from dilutions differing by 10.

The number of bacteria, in practically every case, is estimated "per gram of soil." Probably moist soil is meant in many instances although King and Doryland state that "it is the general custom of bacteriologists to compare the number of bacteria per gram of dry soil." The last mentioned authors express the number of bacteria in soil both as "per cubic centimeter" and "per gram" for purposes of comparison.

Twelve authors state their results definitely on the basis of dry soil (Brown, Chester, Conn, Cook (8), Eyre, Gainey and Gibbs, Heinemann, Hills, King and Doryland, Noyes and Voigt, Remy, and Schneider). Neither Conn, Hills, Schneider nor Noyes and Voigt in the publications here reviewed states how the soil is dried. Eyre dries 10 gm. of soil over a water bath to a constant weight; Gainey and Gibbs dry the soil at 110°C. for 2 hours; Heinemann dries the soil in the hot-air oven 1 hour at 100°C.; and Chester dries the soil for 3 hours at 100°C. On the other hand, Brown, King and Doryland, Cook, and Remy merely air-dry the soil.

This brings to consideration the value of the points in the technic of quantitative bacteriological soil analysis which differ so widely with the different authors.

First, what amount of soil should be employed in making the first dilution? It is well known among soil bacteriologists that in soil, probably more than in any other solid or semi-solid natural medium such as cheese, butter, finely chopped meat, curdled milk, and the like, a localization of physiological groups of bacteria and other microörganisms is a common occurrence. These

localizations do not depend entirely on the fact that the soil is more or less of a solid medium, as do similar localizations or, as it may be termed, colony formations, occurring in more homogeneous media. To the contrary they may depend on the composition of the soil particles with which they are in immediate contact. For example, the *Azotobacter* species is known to colonize around particles of calcium carbonate. It seems, then, that whatever method is employed in mixing the soil sample or however thoroughly it is mixed previous to making the first dilution, a sufficiently large amount should be taken to include colonizations as representative of all desired physiological groups as possible. For this reason samples as small as 1 gm. do not seem to be desirable. Löhnis recognized the importance of this point (23) in obtaining information concerning the transformations in the soil induced by microörganisms. He could not secure satisfactory agreement in duplicates where only 0.5 to 2 gm. of soil were used for inoculation (28). He is inconsistent apparently, however, in recommending the use of 1-gm. samples in the determination of bacterial numbers in soil (21). Noyes and Voigt found in making moisture tests of soils for bacteriological analysis that "it took 10-gm. aliquots to have the duplicates check regularly to 0.1 per cent" and are forced to conclude that "it would take larger aliquots to get good bacteriological results than it would for good moisture results". They recommend 50 gm. of fresh soil as a standard. The type of soil might be used to determine within certain limits the amount taken. In the case of a soil like peat for instance, 100 gm. or more should be taken, depending upon the lack of homogeneity, while with a fine sand a 10-gm. sample might prove representative.

It might be well to obtain more or less of a uniformity in the size of the first dilution for all soils. As noted previously the first dilutions vary with different authors from 1:2 to 1:1000. A dilution of 1:2 may be satisfactory for soils like sand, sandy loam, and the like but for soils having high water-holding capacity, this low dilution would be highly undesirable. The lowest dilution which seems desirable for these latter soils is 1:10, which also gives a satisfactory dilution for the sandy soils, etc. An initial dilution of 1:100 also may prove satisfactory if a sufficiently large amount of soil is taken.

It is previously noted that the various authors have differing ideas of how to go about making a quantitative dilution of soil. Practically all of them take the amount of diluting liquid instead of the soil as a basis in determining the first dilution. Whichever method is correct is apparently a subject of controversy, but should it not be decided one way or the other?

In the lack of homogeneity, soil resembles certain other substances of which quantitative bacteriological analysis is made; for example, hamburger steak, shellfish, cheese and butter. Eyre (9) and Savage (26) obtain the first dilution (1:10) for quantitative bacteriological analysis of oysters, mussels and cockles by placing the comminuted shellfish with their liquor into a 1000-cc. graduate and making up the volume to 1000 cc.

Löhnis (21) dilutes cheese by making up 1 gm. of cheese (ground in a mortar with sand and 10 cc. of water) to 1000 cc.

Butter (13, 21) is diluted by placing 1 gm. into 99 cc. of warm water.

Weinzirl and Newton (30) grind a known weight of meat in a mortar with sand, as is done with cheese, and bring the volume up to the desired dilution.

These methods have been accepted as standard by practically all of the bacteriologists who have occasion to employ them for these or like substances. It seems, then, that soil which is not so markedly different from these other substances should be subjected to the same principle in making the initial dilution, that is, adding sufficient of the diluting liquid to make up the known weight of soil to the desired dilution.

The advisability of grinding the sample previous to making the dilution would appear to depend largely on the type of soil. It would not seem necessary to subject sand, clay and the like to the grinding process, as merely placing it in the diluting liquid would serve to separate the particles sufficiently. Soils containing appreciable amounts of organic matter, however, should be so treated before diluting that the diluting liquid will reach all of the particles in as short a time as possible. This probably would best be accomplished by grinding with a little of the diluting liquid.

Although the size of the remaining dilutions made in quantitative bacteriological soil analysis is important, the ones actually used will depend on the object of the quantitative test.

Right here one point is worthy of mention. Certain authors (Fred, Whiting, and perhaps others) recommend that the first and later dilution of soil be allowed to settle after shaking, before making further dilutions or plating. Does not the same principle hold with solid material to be analyzed as with liquid material? Is it not well known that every effort is put forth by the bacteriologist in making quantitative dilutions of milk to get 0.1 cc. of milk in every cubic centimeter of a 1:10 dilution by shaking thoroughly the dilution flask so that he transfers 0.1 cc. of actual milk in every cubic centimeter of the 1:10 dilution placed in the plate; or does he assume that the vigorous shaking has successfully dislodged the greatest percentage of microbes from the solid portion of the milk which then might be left behind in the flask (if it were possible) as well as not? Curdled milk presents a problem to the bacteriological analyst similar to that of soil. Looking at it in this light, does it not seem very illogical to assume that soil can be washed free of all or even of most of its organisms by the short shaking process employed? Again, are not the facts well known concerning the influence of sedimentation on bacterial numbers, a well-founded argument against allowing the soil to settle in the dilution?

A few authors have recognized this point. Conn (5) advised "care being taken to keep the contents of the flask in motion when any of the suspension was withdrawn." To quote Noyes and Voigt, "get the soil and water in the first bottle * * * thoroughly in motion by shaking and while the mixture is still in motion fill the pipette." Hastings and Wright, Löhnis (21),

and Burgess also state more or less definitely the necessity for keeping the dilution in motion while in the act of transferring portions of it.

The method of estimating numbers of bacteria, either the total count or the enumeration of any one physiological group, is very important. It does not seem presumptuous to state that with soil which fluctuates so much in its moisture content, even when "air-dry," the estimation of numbers should be on the oven-dry basis. Furthermore, it seems logical that the method for oven-drying the soil should be that recommended by the Association of Official Agricultural Chemists (1) in their latest report, i.e., "Dry 2 gm. of the sample . . . . at 100°C. to constant weight." As the bacteriologist generally does not care to subject his soil sample to the pulverizing and sifting process recommended for the chemist, a much larger sample should be used to be representative. The important point is the method of drying. The bacterial content per gram of one sample of undried soil sampled at various times would fluctuate within a wide range due to the fluctuation in the moisture content, and most probably the same thing would be true, only in a much lesser degree, of air-dry soil.

The consideration of these points relative to the quantitative bacteriological analysis of soil completes the purpose of this article. It is not the intent of the writer in this paper to discuss apparatus or methods used in taking soil samples, methods of mixing the sample before making the first dilution, nor the particular dilution liquid, dilutions, or media to use. Most of these latter points have been thoroughly discussed previously by one or more authors with helpful results.

It is certain that most investigators or teachers who have had occasion to deal with quantitative bacteriological soil analysis will appreciate to a considerable degree the fact that the points brought up in this paper are some of the most important of those met with, and that an effort toward a standardization of certain fundamental features of the technic would be a desirable step.

SUMMARY

The following points are suggested as worthy of attention should the standardization of quantitative bacteriological soil analytical methods be considered at any future time.

1. Not less than 10 gm. of soil should be used in making the first dilution.
2. The initial dilution should be not less than 1–10.
3. If the soil contains considerable organic matter, it should be triturated in a mortar with a little of the diluting liquid.
4. In all cases the weighed soil sample should be made up to the volume of the desired initial dilution by the addition of sufficient diluting liquid, e.g., for a 1–10 dilution, 10 gm. of soil should be placed in the graduated flask, cylinder, etc., and sufficient diluting liquid added to bring the volume up to 100 cc.

5. Care should be taken in making further dilutions or in plating to transfer an aliquot of the soil itself, as nearly as possible.

6. The second dilution should contain not less than one-tenth of the amount of soil in the first dilution. Noyes and Voigt's recommendation may well be followed in making succeeding dilutions, i.e., each higher bacterial dilution should be made by taking 10 cc. of the lower bacterial dilution and 90 cc. of the diluting liquid.

7. Numbers of microörganisms (total or otherwise) should be estimated on the basis of soil oven-dried at 100°C. to a constant weight.

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SOME SOIL FUMIGATION EXPERIMENTS WITH PARADICHLORO-BENZENE FOR THE CONTROL OF THE PEACH-TREE BORER, SANNINOIDEA EXITIOSA SAY.[1]

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Since 1917 the author has conducted numerous and varied experiments for the control of the peach-tree borer. During 1918 to 1920 several soil fumigants were tried out. In 1918 and 1919 sodium cyanide was used extensively. The results of the experiments with sodium cyanide may be found in another paper (2). Briefly stated, the results show that sodium cyanide will give fair results under certain conditions. Generally speaking, the margin of safety, that is the difference between the killing point of the larvae and the killing point of the tree, is not sufficient for general practice. Furthermore, sodium cyanide is extremely poisonous to man.

During 1919 and 1920 paradichlorobenzene was used as a soil fumigant for the peach-tree borer. This paper gives a summary of the more important results we have obtained with this product. In November 1919, Blakeslee (1) published the results of his experiments with several soil fumigants. His results with paradichlorobenzene were very encouraging and they have spurred us on to learn all we could about it. The writer has had occasion frequently to refer to Blakeslee's splendid bulletin and it has been decidedly interesting to compare our results with his. For the most part the results of similar experiments agree.

Paradichlorobenzene is a white crystalline substance occurring as a by-product in the manufacture of monochlorobenzene. Paradichlorobenzene is practically insoluble in water. It evaporates slowly at ordinary temperatures (70°F). The gas coming from the crystals is heavier than air. The fumes are non-poisonous to man under ordinary conditions but are toxic to insects when the latter are exposed to them for a considerable period of time. If the directions given in the following pages are followed, paradichlorobenzene will kill 90 per cent or more of the larvae of *Sanninoidea exitiosa*. In a number of experiments 98 to 100 per cent of the peach-tree borers have been killed.

In our soil fumigation experiments with paradichlorobenzene for the control of the peach-tree borer, we have determined or we are determining the following phases of the problem:

[1] Paper No. 19 of the Technical Series, New Jersey Agricultural Experiment Stations, Department of Entomology.

1. The amount of paradichlorobenzene needed to kill all of the small and large borers in trees of varying ages.
2. The effect of varying amounts of paradichlorobenzene on trees of different ages and varieties.
3. The killing effect of short exposures of small and large amounts of paradichlorobenzene on borers in trees younger than 6 years of age, and the response of young trees to short treatments.
4. The best time of the year to make applications.
5. The essential details of the method of application.
6. The influence of soil texture, soil acidity and alkalinity, soil moisture, and soil temperature on the rate of evaporation, and the effectiveness of paradichlorobenzene as a soil fumigant.
7. The cost of applications.
8. Other miscellaneous experiments.

1. STRENGTH OF THE APPLICATION

Our experiments show that prolonged exposures (21 days or more) of $\frac{3}{4}$ and 1 ounce of finely divided paradichlorobenzene per tree, kill 90 per cent or more of the worms present in 6-year old trees provided the soil conditions are favorable. One-half ounce in the vast majority of our experiments gave as satisfactory results as $\frac{3}{4}$ and 1-ounce treatments. Applications of $\frac{1}{8}$ and $\frac{1}{4}$ ounce are insufficient. At their best these strengths did not kill over 50 per cent of the larvae, and the larvae that were killed with $\frac{1}{8}$ and $\frac{1}{4}$ ounces were small, less than $\frac{1}{2}$ inch in length. Large larvae ($\frac{3}{4}$ inch or longer) are more difficult to kill than small larvae. Many of the large larvae are stupefied by small doses or short exposures of the gas, but they are not killed.

2. INJURY TO TREES

Trees 6 years of age or older are not seriously injured by the fumes of paradichlorobenzene if the applications are made when the soil is warm. Slight injury to the outside bark may take place. This usually occurs as a dark brown color in the bark. So far as our experience goes this injury is superficial and of no importance. In a few instances 6-year-old trees or older, which were heavily infested and damaged by borers, died after they were treated with paradichlorobenzene. These trees probably would have died even though they had not received the treatment. In other words, some trees which are damaged and greatly weakened by borers may not be saved by paradichlorobenzene. It is a debatable point whether such trees are killed by the treatment or by the borers. At the present time we are not certain what will happen to 6-year-old trees, or older, if they are subjected to long exposures of paradichlorobenzene when the soil temperature is 55°F. or colder. In November, 1919, we treated 20 trees in two orchards with $\frac{1}{2}$ and 1 ounce applications of paradichlorobenzene. Today these trees appear to

be normal and healthy. In a few cases we have injured (not killed) trees younger than 5 and 6 years of age with prolonged exposures of ½ and 1 ounce of paradichlorobenzene. This is particularly true with trees 3 years of age or younger. Injury appears in the cambium layer as small brown specks, each about the size of the head of a pin. When serious, these spots converge and the entire living layer turns brown, becomes dry and dies.

To date our experiments do not show any variation in the susceptibility of different varieties of peaches to paradichlorobenzene. Only extensive experiments and observations over a considerable period of time can determine this point.

In case prolonged exposures of paradichlorobenzene prove to be satisfactory for trees 6 years of age or older, then it will be necessary to find some method of controlling borers in young trees. It is possible that short exposures of paradichlorobenzene will kill the worms in young trees and will not produce sufficient injury to the tender bark to be of importance.

3. SHORT EXPOSURES

During the past season experiments were conducted in several young peach orchards with the express purpose of determining the killing effect of short exposures (2, 4, 7, and 10 days) of ½ ounce (or greater amounts) of

TABLE 1

Results of 2, 4, 7 and 10-day treatments with 0.5 ounce of paradichlorobenzene on 4-year-old flowering peach trees, in Penn loam soil at Bound Brook, N. J.

Ten trees in each treatment

NUMBER	DAYS EXPOSED	DATE TREATED	DATE REMOVED	LARVAE PER TREE		PERCENTAGE DEAD
				Alive	Dead	
1	*2	October 2	October 4	4.0	1.7	29
2	*4	September 25	September 29	3.1	5.4	63
3	*7	September 15	September 22	0.8	3.3	80
4	*10	September 15	September 25	1.4	3.7	72
5	†2	October 14	October 16	3.4	1.5	30
6	†4	October 14	October 18	2.6	2.9	52
7	†7	October 14	October 21	1.7	4.8	71
8	†10	October 14	October 25	0.5	4.6	90

* Soil temperature 62° to 58° F., moisture 10 to 15 per cent.

† Soil temperature 58° to 56° F., moisture 15 to 20 per cent.

Check trees—larvae all alive.

paradichlorobenzene on borers in trees younger than 6 years of age. The response of the young trees to short exposures also was observed. Table 1 summarizes the results of two series of experiments with heavily infested 4-year-old flowering peach trees subjected to ½ ounce for 2, 4, 7 and 10 days. The table also records the nature and condition of the soil. The 7-day treat-

ments killed 71 to 80 per cent in these experiments while the 10-day treatments killed 72 to 90 per cent. These and other experiments of a similar nature show an average kill of 80 per cent for 10-day exposures with ½ ounce The great majority of the larvae which survived the 10-day treatments were ¾ inch in length or longer. In other words, the small larvae were killed. The author is of the opinion that it is more important to kill the small larvae than the large larvae because a small borer is capable of doing more damage than a full grown (or nearly grown) worm.

Several owners of peach orchards in New Jersey treated their young trees, 3 to 6 years of age, with ½ ounce of paradichlorobenzene in September, 1920. One grower on September 17 treated 125 3-year-old and 600 4-year-old heavily infested Hiley trees with ½ ounce and then removed the crystals and dirt to ground level in 7 days from the 3-year-old trees and in 12 days from the 4-year-old trees. The sandy soil in which the trees were located was warm and somewhat dry during the treatment. Thirty of the trees were examined in October, and an average of 0.1 living worm per tree was seen in the 3-year-old trees and less than 2.0 living worms per tree in the 4-year-old trees. No serious injury was noted; only one 3-year-old tree showed decided injury to the cambium layer about a cavity where a larva had been feeding. Our experiments to date show no serious injury to trees 3 to 6 years of age, where ½ ounce of paradichlorobenzene was used for 7 to 10 days. We have injured nursery stock with ½ ounce with 10 days' exposures, but the seriousness of this injury cannot be determined until 1921. After one or more years' experience with short exposures with varying amounts of paradichlorobenzene more definite statements can be made concerning the effectiveness and safeness of short exposures on young trees.

4. TIME OF APPLICATION—EARLY AND LATE SUMMER TREATMENTS

A series of experiments on the time of application were conducted in five orchards throughout the state. The experiments reported in table 2 were conducted at Bridgeton and the results are representative of all. About one-half of the trees in each series have been wormed; the others will be examined in 1921. All of the trees treated with ½ and 1 ounce on June 25 (exp. 2 and 3) showed no larvae (100 per cent reduction) on July 27, while other trees treated at the same time but examined on October 7 possessed 14 to 15 larvae per tree (exp. 4 to 6). Comparing this infestation with the check trees the reduction approximated 30 per cent. Other trees which received two treatments, June 25 and August 5 (exp. 7 and 8) and were examined on October 7, showed an average of 0.4 or less larva per tree, a reduction of 98 per cent or better. Trees treated September 10 (exp. 9) and examined October 7 showed 99 per cent reduction. This series of experiments and others of a similar nature show that trees receiving two summer treatments or one late summer treatment will be free of borers during the fall, winter, spring and

early summer months, while trees receiving only one early summer treatment in June (or earlier) may possess some or many borers all months of the year except for a period of 6 to 8 weeks immediately following the treatment.

To date we have seen no injury to trees receiving two applications during the summer. Under some conditions two applications may prove to be desirable; however, further experiments and observations are necessary to determine the effect of two applications in one season on different varieties and under varying soil conditions.

Early spring applications are undesirable because the soil temperature is too low for the immediate and effective killing of the borers. Furthermore, the effect on trees of early applications in cold soils is unknown.

TABLE 2

Early and late summer treatments with paradichlorobenzene on Elberta peach trees, 12 years old, in sand to sandy loam soil at Bridgeton, N. J.

NUMBER	NUMBER OF TREES	AMOUNT APPLIED	DATE TREATED	DATE WORMED	LARVAE PER TREE ALIVE	REDUCTION
		oz.				*per cent*
1	10		Check	July 27	1.8	
2	10	1.0	June 25	July 27	0	100.0
3	10	0.5	June 25	July 27	0	100.0
4	5		Check	October 7	22.4	
5	*8	1.0	June 25	October 7	15.8	29.5
6	*5	0.5	June 25	October 7	14.8	34.4
7	*10	1.0	June 25			
			August 5	October 7	0.2	99.1
8	*5	0.5	June 25			
			August 5	October 7	0.4	98.5
9	10	0.75	September 10	October 7	0.2	99.1

* Twenty-five eggs placed on each tree.

In case applications are made after September 10 our present knowledge indicates that it is advisable to pull the dirt away from the trees to ground level where the crystals were placed in order that the gas may get away from the tree before the ground becomes cold and freezes. The dirt may be removed any time after the material has been on two or three weeks provided the soil has not been continuously wet and the temperature has been 55 to 60°F. or higher. In seasons when the weather is warm and dry during September and October most of the paradichlorobenzene will evaporate from the soil even though it is applied as late as September 20 at New Brunswick. To determine the necessity of removing the dirt and the crystals, pull the dirt away from several trees in the orchard before the ground freezes and if no crystals are seen or a strong odor detected it will be unnecessary to remove the dirt.

Our present information indicates that the best time to make an application of paradichlorobenzene is the last week in August or the first ten days in

September, August 25 to September 10. In the average season applications made at this time will kill the borers in the trees, prevent further infestation that season, and give ample time for all or a greater portion of the paradichlorobenzene to evaporate before the ground becomes cold or freezes.

5. METHOD OF APPLICATION

We recommend on the basis of our experiments the following method of application. Prepare the soil for treatment about the base of the tree by removing the grass, weeds and other refuse for a distance of at least one foot. Then make the soil smooth and level for a distance of 6 inches from the tree (pl. 1, fig. 1). Do not dig into the surface crust any more than is necessary, for by so doing numerous large air spaces will be made which are probably undesirable. In case a considerable amount of gum is present about the base of the tree (pl. 1, fig. 4) it is best to remove the bulk of it. It is also advisable to have the surface of the soil where the paradichlorobenzene is applied, level with the highest point on the tree where a considerable amount of gum, containing sawdust-like particles, is exuding. The greatest number of larvae will be killed if this point is observed. We know that the gas coming from the crystals is heavier than air; consequently, if the paradichlorobenzene is placed below some of the larvae in the tree the amount of gas going up from the material is apt to be of insufficient strength to kill the borers above the point of application. In measuring out $\frac{3}{4}$ or 1 ounce of paradichlorobenzene use some vessel which holds, when filled, the required amount. A short wide-mouth bottle, a tin or wooden pill box may answer the purpose.

The paradichlorobenzene is evenly distributed in a narrow continuous circular band on the soil about the tree approximately two inches from the trunk (pl. 1, fig. 2). The band should be about 1 inch wide and no crystals should be closer than 1 inch from the trunk (or large roots), otherwise injury may take place (pl. 1, fig. 5). In case large roots are near the surface of the soil at least one or more inches of dirt should be placed above them before applying the paradichlorobenzene. If the crystals are placed several inches (4 or more) from the tree (pl. 1, fig. 6) the effectiveness of the insecticide is apt to be materially reduced. This is particularly true if the soil is wet.

After the paradichlorobenzene is properly distributed place several shovels of earth (free of weeds, grass, large stones or other refuse) over the "death ring" of paradichlorobenzene and compact it into a cone-shaped pile with the back of the shovel or some other tool (pl. 1, fig. 3). The first shovel of earth placed on top of the treatment should be finely divided and carefully poured upon the paradichlorobenzene. Forcefully throwing the first shovel of earth directly onto the ring of crystals is apt to push some of the material against the tree. This may cause injury. After we learn more about this product we may find that some of the above points may be changed or adherence to some of them may be unnecessary.

6. SOIL CONDITIONS

Our experiments to date indicate that soil texture does not have much influence upon the rate of evaporation. This is particularly true if the soil is dry. Also, soil acidity or alkalinity does not seem materially to affect the rate of evaporation of paradichlorobenzene. The details of the experiments employed in determining the above points are omitted.

Soil moisture

The amount of moisture in the soil has a direct influence on the rate of evaporation of paradichlorobenzene. In our laboratory experiments with four kinds of soil, Penn loam, clay loam, sandy loam and white sand, 1 gm. of paradichlorobenzene evaporated completely in 15 to 16 days when embedded (2 inches deep in 6-inch flower pots) in dry soil and kept at an average temperature of 70°F., while in a similar series where the four soils were kept moist by adding 50 cc. of water each day the 1 gm. of material required four to seven times as many days to disappear completely, the maximum time being 113 days. In other laboratory experiments with varying degrees of soil moisture there was a consistent ratio between the rate of evaporation of the paradichlorobenzene and the amount of water present in the soil.

In several orchard experiments it was noted that moist soils retained the paradichlorobenzene for a greater period of time than dry soils. In one orchard a number of trees were treated with 1 ounce of paradichlorobenzene in June. Some of these trees were on high ground and others on low ground. When the dirt was removed in October the crystals were present and easy to find about the trees in the low ground where the soil had been moist or wet all the season, while no crystals and no odor was present in the soil about the trees on high ground where the soil was much drier. Generally speaking, it requires 6 to 8 weeks for the crystals to evaporate completely and for the gas to disappear when the soil temperature is 60°F. or higher and the soil moisture is low.

We know that paradichlorobenzene is practically insoluble in water; consequently, if evaporation is to take place in the soil it is dependent upon the amount of air in the soil. Water-laden soils possess few or no continuous air spaces, while dry soils are porous containing numerous small air spaces. An excessive amount of water in the soil fills all of the air spaces and thus acts as a barrier to the fumes arising from the paradichlorobenzene.

If the amount of water present in the soil influences the rate of evaporation and distribution of the gas, then one would expect to find that the moisture content of the soil influences the toxicity of paradichlorobenzene as an insecticide for the peach-tree borer. A few experiments have been conducted to determine this point and the results show that fumigation under different degrees of soil moisture produces a decided difference in the percentage of dead larvae when the borers are subjected to short exposures. Seven to ten-

day treatments with ½ ounce in wet (saturated) soils were 40 to 50 per cent less effective than in semi-moist soils where the water content was 25 to 50 per cent less than that of the wet soils (table 3, exp. 1-6).

In the laboratory experiment described below the moisture content of the soil had a decided influence on the distribution of the gas. Two large metal containers (open at the top and the bottom) 12 by 12 by 10 inches were filled with dry sand. The sand in one of these was saturated with water. One-half ounce of paradichlorobenzene was embedded (2 inches deep) in the center in a 2-inch circle in each container. Pieces of fresh peach bark (1 by 3 inches) containing newly hatched larvae (2 to 9 larvae in each piece of bark) were

TABLE 3

Influence of soil moisture and temperature on paradichlorobenzene treatments for the control of the peach-tree borer; trees 4 years of age; moisture experiments 1–6; temperature experiments 7–11

NUMBER	NUMBER OF TREES	AMOUNT APPLIED	SOIL WATER	DATE TREATED AND SOIL TEMPERATURE	DATE "WORMED" AND SOIL TEMPERATURE	LARVAE PER TREE		PER CENT DEAD
						Alive	Dead	
		oz.	*per cent*					
1	*5 (W)	0.5	20±	October 26, 58° F.	November 5, 44° F.	4.4	1.4	24
2	*5 (W)	0.5	15–	October 26, 58° F.	November 5, 44° F.	2.8	4.6	62
3	5 (B)	0.5	30	October 14, 58° F.	October 21, 58° F.	4.4	0.6	11
4	10 (B)	0.5	20	October 14, 58° F.	October 21, 58° F.	1.7	4.8	74
5	*5 (W)	1.0	Wet	June 28	October 19	1.4		
6	*5 (W)	1.0	Dry	June 28	October 19	3.0		
7	10 (W)	0.5	10–15	October 19, 60° F.	October 26, 58° F.	2.0	6.5	76
8	10 (W)	0.5	10–17	November 1, 55° F.	November 11, 44° F.	8.4	1.8	17
9	10 (B)	0.5	17–	November 1, 45° F.	November 8, 48° F.	3.0	0.0	0
10	10 (B)	0.5	17–	November 8, 48° F.	November 22, 41° F.	2.0	0.3	13
11	10 (B)	0.5	17–	November 1, 45° F.	November 22, 41° F.	1.0	0.9	47

* One hundred eggs placed on each tree.

(W) Wolpert Farm, New Brunswick; Sassafras loam soil.

(B) Bound Brook Nurseries, Penn loam soil.

embedded in the soil at intervals of 1, 2, 3 and 4 inches in two directions from the paradichlorobenzene. Other pieces of bark containing larvae were placed in dry and wet sand in flower pots free of paradichlorobenzene. These served as checks. After 3 days' exposure all of the pieces of bark were examined. In the checks all of the larvae were alive. In the container filled with dry sand all of the larvae were dead, while in the container filled with wet sand 10 per cent of the larvae were alive in the pieces of bark 3 inches from the paradichlorobenzene and 40 per cent were alive in the pieces of bark 4 inches from the insecticide. All other larvae in all pieces of the bark in this container were dead. This experiment shows how water may act as a barrier to the gas.

In prolonged treatments made in wet soils the final results may prove to be just as effective as in semi-moist soils. This is expected if the soil becomes somewhat dry sometime after the applications are made and if the soil temperature is 55–60°F. or higher. Further investigation is needed to determine the influence of moisture in long exposures.

Soil temperature

The temperature of the soil also influences the rate of evaporation of paradichlorobenzene. In a number of laboratory experiments where 1 gm. of paradichlorobenzene was embedded (2 inches deep) in each of three flower pots containing dry sand and kept at average temperatures of 68°, 50° and 42°F. for periods of 10 and 20 days, the rate of evaporation in the pot kept at 68°F. was four times as fast as in the pot kept at 42°F. and approximately two times as fast as in the pot averaging 50°F. About the same rate of evaporation held for crystals exposed to open air for 24 hours with the temperatures averaging 70°, 52° and 42°F. Briefly stated our experiments show that high temperatures produce greater rates of evaporation of paradichlorobenzene than low temperatures. If the rate of evaporation varies with the temperature, then one would expect to find that paradichlorobenzene is less effective as an insecticide at low temperatures than at high temperatures.

This effect is shown in a few experiments with short exposures conducted this past season. Table 3 on moisture and temperature experiments shows in one series (exp. 9–11) that 47 per cent of the larvae were killed in 3 weeks when the temperature was 48°F. or less, and in another series (exp. 8) only 17 per cent of the borers were killed by 10 days' exposure at temperatures between 55° to 44°F., while 76 per cent were killed with 7 days' exposure (exp. 7) at 60–58°F. Longer exposures at temperatures of 55° or less might kill a larger percentage of the worms. Further investigation is needed to determine this point.

The above ineffectiveness of paradichlorobenzene at low temperatures probably is not altogether due to the fact that the paradichlorobenzene evaporates more slowly at low temperatures. It is in part due to the fact that at soil temperatures under 55°F. the larvae are inactive and consequently their oxygen requirements are lower.

If low temperatures do not give immediate and effective results, then the question arises, what is the minimum temperature which produces an immediate and effective kill? Our experiments show that the best results with short exposures of paradichlorobenzene are obtained when the soil temperature at a depth of 4 inches under the peach trees registers 60°F. or higher. Fairly satisfactory results have been obtained at temperatures ranging between 55° to 60°F. Below 55°F. the effectiveness and the rapidity of kill with paradichlorobenzene is materially reduced. This is particularly true in short exposures.

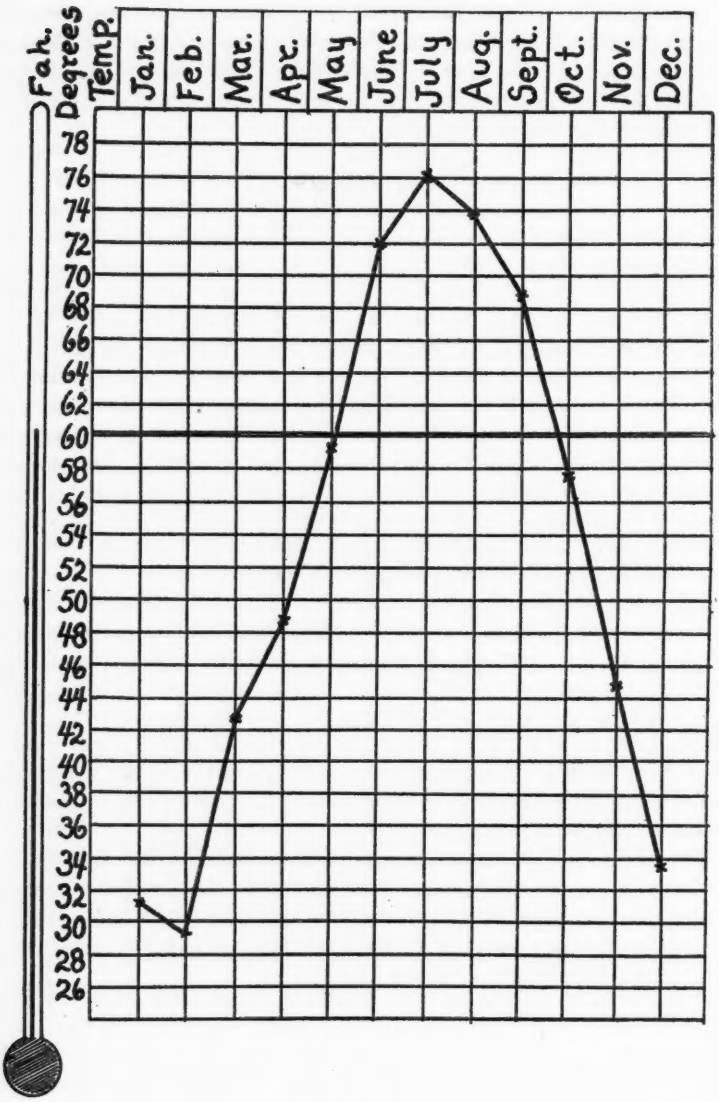

FIG. 1

The plotted line shows the monthly mean soil temperature at six inches for 1898, 1901 and 1902 at New Brunswick, N. J.

The chart with a plotted line shows the monthly average for 1898, 1901 and 1902 of the mean soil temperatures at 6 inches at New Brunswick, N. J. If 60°F. proves to be the minimum temperature for the most satisfactory results with paradichlorobenzene in the control of the peach-tree borer, then at New Brunswick, N. J., an immediate and effective kill of the borer will take place when applications are made between May 15 and October 1, provided the soil moisture is not too great. Earlier or later applications for the average season will not give immediate and effective results because the soil is too cold (fig. 1).

7. COST

Paradichlorobenzene may be purchased from several firms in the United States for 15 to 30 cents a pound, depending largely upon the amount purchased. In several orchards we kept account of the cost of treating trees of various ages under different orchard conditions. Under very unfavorable orchard conditions the greatest cost of a 1-ounce treatment with the material at 20 cents a pound was approximately 4 cents a tree, while in other orchards where 1-ounce applications were more easily made the cost averaged about 3 cents a tree. In one orchard of 800 3- and 4-year-old trees a ½-ounce application, including the removal of the dirt to the ground level in 7 to 10 days, cost 3.5 cents a tree.

8. OTHER EXPERIMENTS

The rate of evaporation of paradichlorobenzene is also somewhat dependent upon the size of the crystals. Various experiments, indoors and outdoors, above and below ground, were conducted with crystals of varying sizes, particularly 3 to 5, 5 to 10, 10 to 20 and 20 meshes (or smaller) to the inch (table 4). One gram or smaller amounts of crystals were placed in glass dishes and exposed to the open air or placed in dry sand (2 inches deep) under inverted glass dishes. The crystals were separated as much as possible in order that the surface of each should be exposed to the air. The rate of evaporation under the above conditions shows conclusively that the large crystals evaporated much more slowly than the small ones. This is expected because a small crystal has a proportionally greater surface than a large crystal. In similar experiments where the crystals were piled on top of each other the rate of evaporation of the crystals of different sizes was more nearly uniform. In field practice the crystals are piled on top of each other, more or less, when placed about a tree, consequently one would not expect a great variation in the rate of evaporation of small and large crystals. This appears to be true where the crystals range from 3- to 20-mesh.

The insecticidal value of large crystals appears to be as great as that of the small crystals. Until we have further information on the influence of the size of the crystals it is believed that they should be 10-mesh or finer. In case the material is lumpy it should be passed through a wire-screen sieve made out

of ordinary window screening or some screen having 10 or more openings to the inch. Finely divided crystals are easy to distribute evenly, and we know that good results may be obtained if they are used.

Some laboratory and field experiments show that full grown larvae of the peach-tree borer in cocoons preparing to pupate and pupae in cocoons may be killed with paradichlorobenzene. The larvae in the cocoons seem to be more susceptible than the pupae. During the fore-part of August, 1920, two lots of 10 and 15 pupae were embedded in sandy loam soil in flower pots (5 inches in diameter) and exposed to weather conditions. Ten and 15 pupae were placed in the center of two pots and 5 gm. of paradichlorobenzene was sprinkled in a circle about them. No crystals were closer than 1 inch from the pupae. Two other pots possessing 10 and 15 pupae served as checks and received no paradichlorobenzene. The pupae and the paradichlorobenzene in all four pots were covered with 1 inch of soil. In September the pupae were examined and 21 out of 25 pupae had emerged in the untreated pots while 1 out of 25 emerged in the treated pots.

TABLE 4

Table showing the number of hours required to evaporate completely crystals of paradichlorobenzene of four sizes .

CONDITIONS	3 to 5-mesh	5 to 10-mesh	10 to 20-mesh	20-mesh
	hours	*hours*	*hours*	*hours*
One gram of crystals placed in glass dish; exposed to room air and average temperature of 80° F.....	48	26	18	12
One-fourth gram of crystals embedded 2 inches in dry sand in flower pots (5 inches in diameter), exposed to room air and average temperature 70 to 80° F.....	190	153	126	105

Another experiment with pupae and paradichlorobenzene was started on August 25 outdoors in the ground at the laboratory. Individual pupae (20 altogether) were embedded 1 inch deep in sandy loam soil at intervals of 1, 2, 3, 4 and 5 inches away from a 2-inch circle of ½ ounce of paradichlorobenzene embedded 1 inch in the ground. There were four lines of pupae at right angles to each other, making a total of 4 pupae at each distance. On September 20 the experiment was observed and 18 of the 20 pupae had emerged. One pupa at 1 inch and one at 2 inches failed to emerge. This experiment indicates that the pupae were not affected by the paradichlorobenzene. There seems to be a decided difference in the results of the above experiments with the pupae.

It is possible that the fumes of the gas were much stronger in the flower pots than in the ground outdoors. Furthermore, in the flower pots the pupae were inside of a circle of paradichlorobenzene. The fumes are undoubtedly much stronger inside a small circle than on the outside of a mass. Unfortunately the moisture conditions of the soil in the above experiments were

unknown. Probably the soil in the pots exposed to the wind and sunlight was much drier at times during August than the ground soil. Further experiments are needed to determine the influence of paradichlorobenzene on the pupae of the peach-tree borer.

Other experiments have been conducted on other phases of soil fumigation with paradichlorobenzene, but at this time the information is insufficient to report.

CONCLUSIONS

Paradichlorobenzene gives considerable promise of becoming a valuable and an important insecticide for the control of the peach-tree borer. The use of paradichlorobenzene for the control of the peach-tree borer is still in the experimental stage; however, we feel that sufficient information has been secured to warrant an announcement concerning its possibilities as an insecticide for the most destructive peach-tree pest. It is suggested that peach growers try out this material for a year or two on a small block of trees 6 years of age or older before treating their entire planting of old trees.

Paradichlorobenzene for the peach-tree borer is not a "fool-proof" remedy, consequently there are certain points in its use which must be carefully followed. Briefly stated, $\frac{3}{4}$ to 1 ounce of paradichlorobenzene will kill 90 to 100 per cent of the borers if the soil temperature is 55 to 60°F. or higher, and the soil is not too wet. It can be used with a considerable margin of safety on trees 6 years of age or older. In making applications the finely divided crystals are evenly distributed in a narrow continuous circular band on smooth ground about the base of the tree. The band should be approximately 2 inches from the tree and no crystals should be closer than 1 inch from the tree. In case there are indications of borers in the tree 1 to 6 inches above the ground, the best results are obtained if the soil is mounded about the tree so that the upper level of the soil is even with the highest point where the gum, containing saw-dust-like particles, is exuding from the tree. The application should then be made on the new soil level. After the paradichlorobenzene is properly distributed place several shovels of dirt, free of weeds, grass, large stones, sticks, etc., over the "death ring" of crystals and pack down the dirt with the back of a shovel or some other tool. For New Jersey conditions the best time of the year to make applications is during the last week in August or the first 10 days in September, August 25 to September 10.

ACKNOWLEDGMENT

The writer is indebted to a number of owners of peach orchards in New Jersey for coöperation in this investigation. He also wishes to thank Dr. T. J. Headlee, Dr. J. G. Lipman and other individuals who have given many valuable suggestions and information pertaining to the problem.

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PLATE 1

FIG. 1. The soil about the peach tree made smooth and ready for treatment.

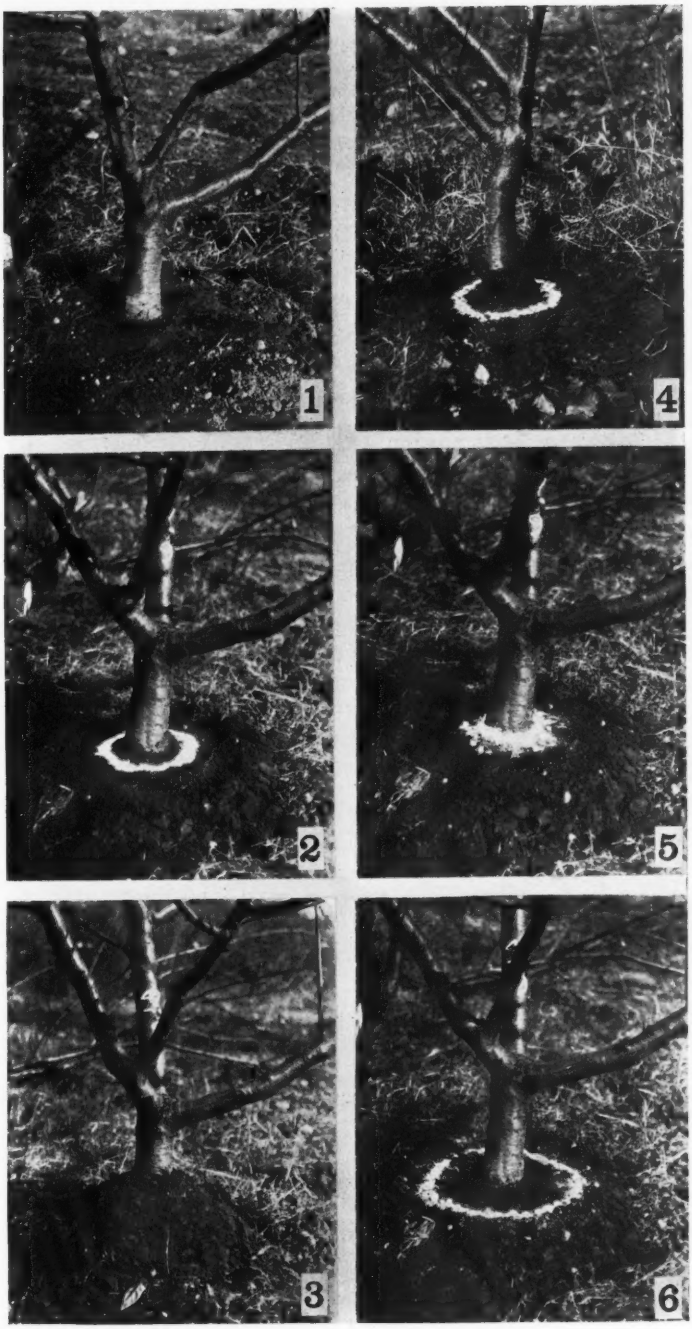
FIG. 2. One ounce of finely divided paradichlorobenzene placed on the soil about the tree in a continuous narrow band approximately 2 inches from the tree; correctly applied.

FIG. 3. Dirt free of grass, large stones, sticks, etc. placed on top of the paradichlorobenzene 4 to 6 inches deep and packed down.

FIG. 4. Paradichlorobenzene incorrectly applied; note the gum exuding from the tree 4 inches above the soil level; dirt should have been placed about the tree to a level equal with the highest point where the gum is exuding before the application was made.

FIG. 5. Paradichlorobenzene incorrectly applied; if the material is placed against the trunk serious injury or death may take place; all crystals should be at least one inch from the tree; also note the large lumps; these are undesirable.

FIG. 6. Paradichlorobenzene incorrectly applied; in this figure the material is placed too far away (6 inches) from the tree to be effective as a killing agent for all of the borers.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THE EFFECT OF CONTINUOUS CROPPING UPON THE MAJOR SOIL NUTRIENTS

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Thirteen samples of California soils from sections of the state as widely separated as the grain lands of the northern Sacramento Valley and the orange groves of Riverside were collected and brought to the Agricultural Experiment Station at Berkeley in 1915. For six seasons these have been the basis for a series of intensive studies of the changes that occur in soil nutrients as the result of the growth of successive crops of cereals.

The first criterion in the choice of these samples was similarity of texture. Six of the thirteen were silty clay loams, collected from different areas, but all, according to the maps of the United States Bureau of Soils, members of the same soil series. These six, then, displayed similarity not only in the texture of the soil, but in their color and general appearance as well. The remaining seven were fine sandy loams. The members of this group were chosen so that each of them represented a different soil series, derived from a different geological formation. While their texture was similar, there was considerable variation in their color and general appearance.

In order to avoid the differing seasonal and climatic influences to which they would have been subjected in their normal environments, the soils were brought to Berkeley in 2-ton samples. Here each sample was divided between duplicate containers holding approximately 1800 pounds.

For the first season, all the containers were planted to Beldi barley. In the succeeding years, one container of each soil has been cropped with this same grain, while the other has been left unplanted. During this time the soils have been quite removed from the leaching effect of rainfall or of excessive irrigation. During the growing season the moisture content of the planted and unplanted containers alike has been maintained at the optimum by the daily addition of distilled water.

We have had available then, under the condition of continuous cropping and that of continuous fallowing, these two sets of soils, of which the members of one set were united by the closest possible relationship, while the members of the other were of exceedingly diverse origin. It has been possible, therefore, to draw between the members of each set, conclusions as to chemical differences in composition and in crop production.

Elsewhere there have been reported the earlier portions of the work dealing with seasonal studies of the water extract (4) and of the freezing-point depres-

sion (2), as well as comparisons of the chemical composition (1). For the purposes of this article it will be sufficient to state that differences of productivity in either set of soils were not associated with corresponding differences in the total chemical composition as estimated by any of the usual methods; that the most adequate criteria of such differences appeared to be the seasonal studies of the water extract, reflecting as they do the changes in the soil solution, and the expression of the total of these changes by the freezing-point depression of the soil.

By means of these methods of procedure striking differences were observed between the planted and unplanted soils, and also between the individual soils. It was clearly shown that the water-soluble nitrates, calcium, potassium, and magnesium present in the soil were notably reduced by the growing crop. Great dissimilarities in the phosphate content of the different soils were observed, although the water-soluble phosphates did not at first show appreciable changes resulting from the growth of the crop.

The details of these results are given in the papers previously issued.

Under continuous cropping, these soils, as might be expected, have notably decreased in crop production. They have not yet reached a constant level. The average decrease of the total crop amounts to 34.9 per cent, that of the straw to 35.2 per cent, and that of the grain to 34.4 per cent.

The study of the soil nutrients in the continuously planted soils has developed certain interesting facts. Observations of the phosphates, during the earlier seasons, did not reveal an appreciable reduction of this constituent as a result of the growing of a crop. But a comparison of the results of five years discloses the fact that in five of the seven sandy loams there has been a reduction of 30 per cent or more in the content of soluble phosphates, while only two of the silty clay loams have begun to show a decrease in this nutrient. There is no indication, so far, that the lowering of the phosphate content accounts for the first decrease in the crop yield; for most of the soils whose soluble phosphates have been reduced are at the present time producing the best crops both of grain and of straw.

Determinations made before the soils were planted showed that the fine sandy loams contained from 0.05 to 0.09 per cent, and the silty clay loams from 0.13 to 0.18 per cent of total nitrogen. Of this nutrient there has been a continual loss. At the close of the season of 1919 all of the soils, both planted and fallow, showed a reduction of from 14 to 38 per cent of their original total nitrogen content.

Investigations of nitrogen losses have generally been made under humid conditions, where drainage has been a large factor in the disappearance of nitrates. Shutt (3) and a few other workers have studied the decrease of nitrogen in prairie soils subjected to little excess rainfall. Their results show that the crop removed only one-third of the nitrogen lost.

In the experiment here described, where the soils have never been subjected to leaching from rainfall or over-irrigation, each season's crop removed

about 17 gm. of nitrogen from each container, while the annual loss from the same soil amounted to about 60 gm. It should be noted, also, that the nitrogen loss from the uncropped soils was almost as great as was that from the planted containers. This constitutes an interesting example of the decrease of our most valuable soil constituent through the ordinary processes of cultivation and tillage. It illustrates, too, the decrease in soil productivity resultant upon a one-crop system of grain farming.

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